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Brit. J. Ophthal. (1954), **38**, 385.

COMMUNICATIONS

PATHOLOGICAL BASIS OF RETROLENTAL FIBROPLASIA

BY

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SINCE the pathological picture of the final stage of retrolental fibroplasia is not of a specific character, no important advance in our understanding of the disease was to be expected until the distinctive histological features of its earliest phases became known. Following a report on the clinical appearances of the developing disease (Owens and Owens, 1949), there soon appeared a number of careful microscopical studies which provided a clear concept of its pathogenesis; from the histological evidence accumulated in the works of Heath (1950a, b; 1951), Tyner and Frayer (1951), Reese and Blodi (1951), and Reese and others (1952), it became abundantly clear that the primary pathological change is angioblastic in nature and presents as an overgrowth of the developing retinal vessels. Some of the conclusions reached in these studies, however, differ in detail from our own and, while it is no longer necessary to present a detailed study of the histology of this condition, it would appear desirable to report upon the pathology of a few representative cases from our own material and to discuss it in relation to the observations already in the literature.

The features of the late stages of the disease are generally agreed upon and have already been fully described (Reese and Payne, 1946; Krause, 1946; Klien, 1949; Wolff, 1950); this report, therefore, is mainly concerned with the earlier stages of the disease and summarizes the pathological basis of our experimental work reported elsewhere in this issue (Ashton and others, 1954; Ashton and Cook, 1954).

MATERIAL AND FINDINGS

CASE 1 (R.L.F. 31), FEMALE INFANT (case of Dr. E. Hinden).—The mother was admitted to hospital at 24 weeks with pre-eclamptic toxaemia and chronic nephritis. This was her seventh pregnancy (the other children being healthy). Caesarian section was performed; the baby appeared normal at birth which was 12 weeks premature. The birth weight was 2 lb 8 oz (1,134 g.). The infant was placed in an oxygen tent from birth until about the fifth week, and was breast-fed throughout. A blood transfusion was given at the seventh week (150 ml.). The infant died when 7 weeks old.

Ophthalmological Appearances (Dr. M. Klein).—In the fourth week there were no abnormal findings, but a few days before she died there was an extensive greyish reflex from the temporal periphery of each fundus without definite detachment, and retrolental fibroplasia was suspected.

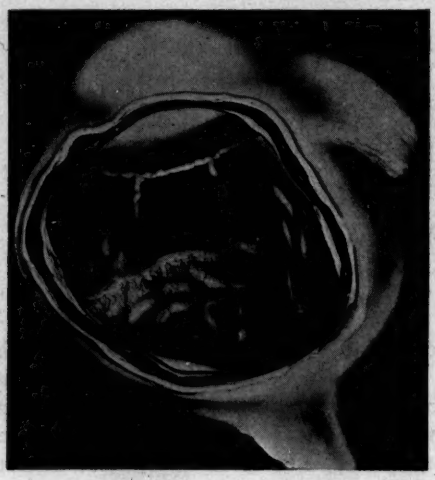

FIG. 1.—Case 1, globe opened horizontally. Arising from the retina in the equatorial zone on the temporal side there is a dense outgrowth of vessels terminating in arborizing tufts buried in yellow gelatinous material.

Autopsy Findings (Dr. M. S. Ross).—Cardiac failure, broncho-pneumonia, and anaemia. Both eyes were removed for examination.

Pathological Examination.

Right Eye.—The globe was opened horizontally to reveal a clear vitreous and very striking retinal changes (Fig. 1). Arising from the retina in the equatorial zone and most markedly on the temporal side, there was an extensive and dense outgrowth of new vessels advancing towards the ora serrata. They formed a wide well-demarcated band around the temporal side of the equator; each vessel growing out from the retinal tissue terminated in aborizing tufts which were buried in yellow gelatinous material. The underlying retina was oedematous and showed an early localized folded detachment. Scattered throughout the retina elsewhere, but mainly equatorially, there were isolated tufts of vessels growing out of the retina and these also were surrounded with gelatinous material. Photographs and drawings were made of the specimen and of flat preparations of the retina (Figs 2, 3, and 4); carbowax and serial celloidin sections were cut and stained by haematoxylin and eosin, Masson's trichrome stain, periodic acid-Schiff stain, and Holmes' silver stain; the carbowax sections were also stained with Scharlach R. and iron-haematoxylin.

Sections.—The globe was normal in size, and the cornea, corneo-iridic angle, iris, and lens were normal. There was a mild diffuse chronic inflammatory infiltration throughout the ciliary body and choroid, and scattered focal aggregations of lymphocytes in the posterior choroid. The vessels throughout the uveal tract were engorged. The predominant pathological changes, however, were to be seen in the retina, which at the equator on the temporal side was folded, shallowly detached, thickened, and oedematous. In this area there was a profuse outgrowth of large, dilated, new vessels, which apparently emerged from the inner layers of the retina and extended into the vitreous; their growth was preceded by a solid cellular mass of vaso-formative tissue in which the new vessels were developing (Fig. 5). This cellular tissue corresponded to the yellow gelatinous material seen macroscopically. Between the retinal surface and the advancing layer of vaso-formative cells there was an irregular space, traversed by the newly-formed vessels and containing a fine, fibrillar transudate in which several large endothelial cells could be seen, many of them containing refractile globules. Adjacent to this area and at other scattered points

FIG. 2.—Case 1, flat preparation of untreated retina, showing vessels growing from the surface into the vitreous. ×22.

throughout the retina, earlier stages of this process could be seen; they consisted of foci of vaso-formative tissue within the nerve fibre and ganglion cell layers. Elsewhere, and particularly anteriorly, there was a diffuse proliferation of spindle cells associated with numerous PAS-positive granules, an association absent from the fully formed endothelial cells. It was interesting to note that within the retina the vaso-formative tissue was confined to the ganglion cell and nerve fibre layers and did not invade the retina beyond the inner molecular layer. On both sides of the globe there was an oral, folded detachment of the retina, which was more marked on the nasal side where the detached retina was thrown into several convolutions. In some sections a very early separation of the pars ciliaris retinae could be seen, but in no section was this extensive.

FIG. 3.—Case 1, flat preparation of untreated retina, showing a tuft of new vessels growing into the vitreous from an intra-retinal vessel. ×22. (Cf. Figs 11 and 15, Ashton, Ward, and Serpell, 1954).

FIG. 4.—Case 1, flat preparation of untreated retina, showing a characteristic glomerular vessel growing into the vitreous. ×206. (Cf. Fig. 11, Ashton, Ward, and Serpell, 1954).

FIG. 5.—Case 1, section of equatorial retina showing a profuse outgrowth of large dilated vessels which emerge from the inner layers of the retina and extend into the vitreous; their growth is preceded by a solid cellular mass of vaso-formative tissue. Haematoxylin and eosin ×36. (Cf. Fig. 17, Ashton, Ward, and Serpell, 1954).

Throughout the retina there was a patchy absence of the internal limiting membrane, and areas of degeneration could be seen in the stratum opticum. Fibrinous strands containing endothelial cells extended from the retina into the vitreous, particularly from those areas in which new vessel formation had occurred. The rods and cones showed *post-mortem* degeneration and were partially replaced by eosinophilic fluid. The remainder of the retina, optic nerve, and sclera appeared normal. Carbowax sections showed no evidence of fat in the retina or choroid.

Left Eye.—The globe was opened horizontally. Situated in the temporal half in the region of the equator there was an irregular band of new vessel formation growing forwards into the vitreous; each vessel had broken up into a number of terminal twigs which arborized with each other and were buried in a mass of yellow, gelatinous material. The retina was thrown into a number of folds in this area and showed a greyish-white appearance as far forward as the ora serrata. The nasal half of the retina appeared normal. The vitreous contained a few fibrinous bands. The specimen was retained for museum purposes and no sections were cut.

Diagnosis.—*Retrolental fibroplasia. Pathological Stage I (bilateral).*

CASE 2 (R.L.F. 31), FEMALE INFANT (case of Dr. Mary Crosse).—This infant was 12 weeks premature, with a birth weight of 2 lb (907 g.); she was the second of triplets (one having died before admission, and the other being alive and well with no blindness). She was given oxygen for 43 days, the concentration starting at 45 per cent., with reduction after the first 10 days first to 40 per cent. and then to 30 per cent. She was in the oxygen tent for 37 days and was then replaced on 76th day for 6 days (pneumonia). Eight small blood transfusions were given. Breast feeding was followed by artificial feeding. The eyes were examined on the 51st day and appeared normal. The baby died when 82 days old.

Autopsy Findings. (Dr. H. S. Baar).—Broncho-pneumonia, severe active rickets, no congenital abnormalities. Both eyes were removed for examination.

Pathological Examination

Left Eye.—The globe was opened horizontally. Arising from the equatorial region superiorly and on the temporal side a number of fine strands extended from the retina into the vitreous and terminated in a solid white band of tissue.

Sections.—The cornea, corneo-iridic angle, and lens were normal. The iris, ciliary body, and choroid showed a mild infiltration with chronic inflammatory cells. In its anterior half, the retina showed a marked proliferation of spindle cells and endothelial cells in the stratum opticum. The spindle cell proliferation was diffuse and particularly well marked in the retinal periphery, whereas the endothelial cell proliferation was focal and more closely related to blood vessels. At the equator on the temporal side new vessels could be seen extending from the retina into the vitreous where they merged with a mass of vaso-formative tissue (Fig. 6). The vitreous was albuminous and contained fibrinous

FIG. 6.—Case 2, section of retina showing marked proliferation of mesenchymal cells in the inner retinal layers and a mass of vaso-formative tissue consisting of endothelial cells and new blood vessels extending into the vitreous. Haematoxylin and eosin. ×90.

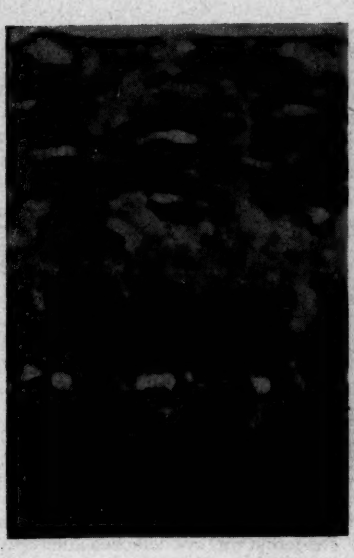

FIG. 7 (*a*).—Case 2, section of retina showing spindle-shaped mesenchymal cells in the inner layers of the retina. Haematoxylin and eosin. ×100.

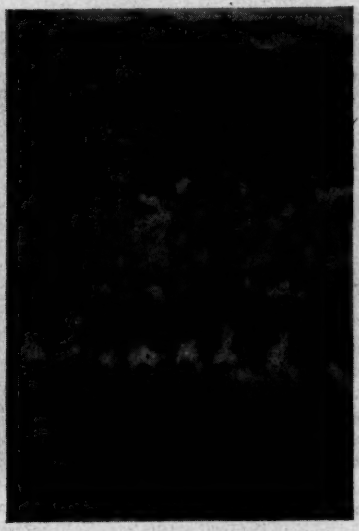

Fig. 7 (*b*).—Case 2, section of similar area stained with PAS showing association of mesenchymal cells with PAS-positive granules. Haematoxylin and eosin. ×100.

bands and occasional haemorrhages. The remainder of the retina showed a moderate degree of oedema but there was no evidence of *ante-mortem* detachment at any point. PAS staining showed abundant red granules confined to the region of spindle cell proliferation within the anterior optic nerve fibre layer on the temporal side (Fig. 7).

Right Eye.—Macroscopically and microscopically the eye was remarkably similar.

Diagnosis.—*Retrolental fibroplasia. Pathological Stage I (bilateral).*

CASE 3 (R.L.F. 32), MALE INFANT (case of Professor R. W. B. Ellis). The birth was 8 weeks premature, the birth weight 3 lb 9 oz (1,616 g.), and oxygen was given (details not available). There were no blood transfusions. The infant was admitted to hospital with retrolental fibroplasia at the 10th week. He was treated with cortisone for 6 weeks, but died suddenly and unexpectedly at the 16th week.

Autopsy Findings (Dr. A. R. MacGregor).—No abnormality was found and it was thought that death was in some manner related to the administration of cortisone.

Pathological Examination

Left Eye.—The globe was opened horizontally. Postero-inferiorly there was a large retinal detachment, upon the surface of which new-formed vessels could be seen. Many vascular and fibrous strands extended from the detached area to the posterior surface of the lens. The newly formed vessels appeared to be empty. A few haemorrhages were present in the retina.

Sections.—The cornea, corneo-iridic angle, iris, and lens appeared normal. On one side there was a focus of inflammatory cells in the ciliary body. In this area extensive haemorrhage had occurred on the retinal surface, and numerous newly formed vessels and organizing fibrous strands could be seen to extend into the vitreous in company with irregular masses of vaso-formative tissue from which fibroblasts arose. Here the retina was detached and a subretinal exudate containing fibrils and macrophages was present. Elsewhere the retina showed focal aggregations of proliferating endothelial cells and diffuse hyper-

FIG. 8.—Case 3, section showing vaso-formative tissue and fibroblasts invading vitreous. Haematoxylin and eosin. ×112.

plasia of spindle cells in the stratum opticum. In general the intensity of the reaction diminished as the ora serrata was approached. The retina was not detached anteriorly and there was no separation of the pars ciliaris. PAS staining showed dense aggregations of positive-staining granules in those areas where spindle cells were proliferating in the inner layers of the retina, but not in the areas of endothelial proliferation or new vessel growth.

Right Eye.—The globe was opened horizontally. Situated posteriorly and to the nasal side, there was a folded retinal detachment covered with extensive haemorrhage, and small haemorrhages were present elsewhere. An extensive net of newly formed vessels with fibrous strands extended into the vitreous and was adherent to the adjacent retina and posterior surface of the lens.

Sections.—The cornea, corneo-iridic angle, and lens were normal. There was some albuminous exudate in the anterior chamber and the iris showed an increased cellularity. On one side there was commencing elongation of the ciliary epithelium. Posteriorly there was a folded detachment of the retina which extended almost to the ora serrata but did not involve the pars plana. A subretinal exudate containing distended macrophages was present, and within the folded retina there was a focal proliferation of endothelial cells and a diffuse proliferation of spindle cells. Masses of vaso-formative tissue extended through the internal limiting membrane, and from this area new vessels and strands of fibrin and fibroblasts invaded the vitreous (Fig. 8); there were a few adjacent haemorrhages. Elsewhere the retina showed spindle cell and endothelial proliferation in the inner layers, and there were small turrets of fibrin attached to the internal limiting membrane. The sclera and optic nerve were normal; many PAS positive granules could be seen in association with the spindle cells.

Diagnosis.—*Retrolental fibroplasia. Pathological Stage II (bilateral).*

CASE 4 (R.L.F. 35), MALE INFANT, FIRST CHILD (case of Mr. A. L. M. McCurry).—The birth was 11 weeks premature, and the birth weight 2 lb 6 oz (1,077 g.). He was nursed in an oxygen tent for 26 days from the time of admission to hospital; oxygen concentrations were not recorded but the flow was about 4 l./min. Artificial feeding was given throughout and no blood transfusions were given. The eye condition developed when he was approximately 1 month old, and he was given ACTH 5 mg. 6-hrly for about 6 days, but, as the eyes became worse, this was increased to 6.25 mg., and continued for 6½ days. At the end of this time the eyes were beginning to improve, but the baby had become grossly oedematous and treatment was discontinued. He died 24 hrs later.

Autopsy Findings (Dr. J. N. Dearnaley).—Apart from gross oedema no abnormality was found. Both eyes were removed for examination.

Pathological Examination

Right Eye.—The globe was opened horizontally; the vitreous was cloudy and contained numerous white strands which appeared to extend from the scarred retinal surface.

Sections.—Pupillary membrane remnants were present, the choroid was congested, and white blood cells were visible in the vessels, but no cellular infiltration was present. The retina was folded posteriorly, but there was no oral fold and no retinal detachment. In

FIG. 9.—Case 4, section showing diffuse proliferation of mesenchymal cells in inner layers of anterior retina. More posteriorly there are discrete masses of endothelial cells which at one point have broken through the internal limiting membrane. Haematoxylin and eosin. ×50.

FIG. 10.—Case 4, section showing mass of endothelial cells erupting through internal limiting membrane. Haematoxylin and eosin. ×115. (Cf. Fig. 13, Ashton, Ward, and Serpell, 1954).

FIG. 11.—Case 4, section showing mass of endothelial cells growing along the retinal surface beneath the hyaloid membrane of the vitreous. Haematoxylin and eosin. ×115.

the anterior half of the retina, where there was no invasion of the vitreous, a diffuse proliferation of spindle cells could be seen in relation to the capillary bed, while further posteriorly the endothelial cells tended to form discrete masses (Fig. 9). In the posterior half of the retina and especially near the optic disc a number of endothelial masses had broken through the internal limiting membrane (Fig. 10) and others were growing along the surface of the retina beneath the hyaloid membrane of the vitreous (Fig. 11). PAS-positive granules were present in relation to the proliferating spindle cells, but only a few such granules were present in the endothelial masses. Despite the ACTH therapy the disease process appeared active.

Left Eye.—Macroscopically and microscopically the left eye showed essentially the same changes, but invasion of the vitreous was more marked.

Diagnosis.—*Retrolental fibroplasia. Pathological Stage I (bilateral).*

Discussion

The earliest histological change which can be distinguished as a disease process in retrolental fibroplasia is an excessive proliferation in the inner layers of the retina of vaso-formative tissue, which breaks through the internal limiting membrane, creeps along the surface of the retina beneath the hyaloid membrane, and then invades the vitreous proper. Through the freely permeable angioblastic tissue, protein transudates seep into the retina and also into the vitreous. The oedematous retina becomes folded, distorted, and shallowly detached; meanwhile fibroblasts proliferate in the plasma matrix within the vitreous and form fibrous strands, which on contracture complete the retinal detachment; the fibrovascular tissue thus comes to lie behind the lens, where it further organizes into a dense retrolental fibrous membrane. The cases described in this paper illustrate the above sequence of events and our findings are in agreement with those of Friedenwald and others (1951) and Reese and others (1952).

As in the series described by the last-named workers, the oral region was not maximally involved in any of our cases; exuberant vaso-formative tissue may extend into the ora in the later stages of the disease, but in our experience it has never been the primary site affected. Nor is this surprising, since vaso-formative tissue has not reached the extreme periphery in the more susceptible group of infants of younger gestational age. Indeed, the oral region is the area most frequently found to be normal in the initial stages of the disease, a fact which again emphasizes the angiomatous nature of the process and the absence of glial reaction. Our findings in this respect are, therefore, the reverse of those of Heath (1951), who concluded that the primary retinal disease occurred in the incompletely differentiated retina at its oral attachment and that the denser tissues elsewhere in the eye escaped the oral changes.

In retrolental fibroplasia it has frequently been observed that the retinal detachment may extend beyond the ora serrata to involve the pars ciliaris retinae, a point particularly emphasized by some workers (Dixon and Paul, 1951) and even thought to be of aetiological importance (Wolff, 1950; Bembridge, 1951). While this feature has also been noted in some of our cases (*e.g.* Case 1), its importance is now thought to have been over-stressed, for an examination of premature eyes has shown that the pars ciliaris retinae is not firmly coapted (Ward, 1954), and consequently readily separates with detachments of the pars optica retinae. No specific significance can, therefore, be given to such anterior detachments in the developing eye.

Within the inner layers of the retina in the early stages of the disease there is, in addition to the hyperplastic foci of endothelial cells, a diffuse pro-

liferation of spindle cells which had been noted by Friedenwald and others (1951), and by Reese and others (1952), who described them as " glial cells ". However, when these cells are studied as regards distribution, occurrence, and staining properties, it becomes apparent that they are not of glial origin. They are not present in the normal retina before vascularization commences, they enter with the vascular ingrowth immediately ahead of the advancing vascular buds, and they are similarly confined to the inner layers of the retina; as the endothelial cells multiply and canalize into vessels, so the spindle cells disappear and are again absent in the mature retina. They are always intimately associated with PAS-positive granules, which have been identified as glycogen (Serpell, 1954), whereas fully-developed endothelial cells are never so associated. These features are not the characteristics of retinal glial cells, and the only explanation of their nature which appears to be consistent with all the facts is that they are mesenchymal precursors of the retinal vascular system. Indeed, such mesenchymal tissue would be entirely necessary for the formation of the other vascular components, such as connective and muscle tissue, which presumably could not derive from a simple endothelial ingrowth. They probably originate from the mesodermal coats of the hyaloid artery and grow into the retina as solid cords which canalize and differentiate into endothelium and eventually form the complete vascular network, a process exactly comparable with the formation of the closed vascular system elsewhere in the body. In future, therefore, we shall refer to them as mesenchymal cells; they are discussed in greater detail by Serpell (1954).

In the sections of our cases no new vessels were found on the iris; this is somewhat surprising, for their clinical appearance in the active stages of retrolental fibroplasia has been described by a number of writers; Von Winning (1952) for instance described and illustrated convoluted tufts of engorged vessels at the pupillary margin. Such proliferations have also been produced in the iris experimentally (Ashton and others, 1954), but they were found to occur inconstantly and to disappear rapidly, and they are not readily demonstrable in sections unless one is fortunate enough to obtain an eye at the right stage and to cut through the particular area involved. That vascularization of the iris may be demonstrated in sections of eyes with retrolental fibroplasia has been shown by Reese and Blodi (1951).

It has been claimed that the early stages of retrolental fibroplasia may occur pre-natally, since proliferative changes have been noted in the retina in stillborn infants, and in premature infants dying soon after birth. It is to be remembered, however, that the developing retina of the normal foetus must show some degree of endothelial and mesenchymal proliferation as part of the process of retinal vascularization. Thus the matrix of retrolental fibroplasia is present in the normal developing retina, and the histological diagnosis of the early stages of the disease will depend upon the degree of proliferation regarded as excessive. Here we are at the boundaries of normal and abnormal activity, and, although proliferations are occasionally seen which are more

exuberant than is commonly found, it cannot be said with certainty that they are beyond the limits of normal variation. No departure from normal can confidently be assumed until the vaso-formative tissue appears in an abnormal situation, either extending into the vitreous or proliferating to a marked degree in posterior regions, where, in the last months of intra-uterine life, the formation of the vascular network should be nearing completion.

In order to avoid doubtful diagnoses, we have taken the former criterion as an indication of a definite disease process, and we regard the first pathological stage of retrolental fibroplasia as the early extension into the vitreous of vaso-formative tissue which has erupted through the internal limiting membrane (Fig. 10). When this strict criterion is adopted many of the proliferative retinal changes described in the literature become of doubtful or no significance, and it is of interest that in none of our cases of stillborn and short-lived premature infants, has a Stage I pathological change been observed. It is true that Reese and others (1952) described a stillborn infant showing typical vascular proliferations into the vitreous, but this appears to be the only well-authenticated case in the literature, and it cannot be said that it has yet been established that retrolental fibroplasia, as generally understood, may occur pre-natally. Neither have we encountered, in our whole collection of about 280 cases of retrolental fibroplasia investigated clinically or histologically (or both), a single example of the disease where oxygen had not been administered therapeutically. Nor have we seen the disease in a fully mature infant. This is not to deny, however, that the disease may occur pre-natally or may develop apart from prematurity or oxygen therapy; since the basis of the disorder is merely an aberrant overgrowth of developing retinal vessels, it is not improbable that there may be a variety of precipitating causes, as in the case of retinitis proliferans, which is an approximately comparable process in the adult retina; some of these factors may operate pre-natally, as in congenital encephalo-ophthalmic dysplasia, or post-natally when the retina develops ischaemic anoxaemia by some means other than through oxygen vaso-obliteration. Nevertheless, cases of retrolental fibroplasia of the type which has emerged as a clinical entity during the last 10 years, occur in premature infants who develop the disease post-natally in circumstances related to oxygen administration. Hence, while related conditions may be exactly similar histologically, they are not identical aetiologically, and there would appear to be good grounds for applying a special name to the oxygen-induced disease. This is not expressed satisfactorily in the terms " retrolental fibroplasia ", " retinopathy of prematurity ", or " hypoxic retinopathy " ; it is probable, however, that a revision of the nomenclature, although clearly desirable, should await further clarification of the problems of pathogenesis. It is for this reason that we have retained the familiar name of " retrolental fibroplasia " throughout our papers, realizing that it describes only the final non-specific phases of the disease, that it may embrace a number of different entities, and that it may eventually give place

to a more accurate classification.

Our conclusions are, therefore, in agreement with those of most workers, in that the pathological evidence is in favour of regarding retrolental fibroplasia as a primary retinal disease of non-inflammatory origin resulting from a derangement of retinal vascularization. The intravitreal growth is an extension of the abnormal angioblastic activity in the retina and does not indicate a primary disease of the vitreous as suggested by Reese (1952). Indeed, we believe that the stimulating factor which attracts the vessels into the vitreous is elaborated in the retina. The development and course of the disease may be simply stated in the following pathological stages:

Stage I.—Proliferation of vasoformative tissue, consisting of endothelial and mesenchymal cells in the inner layers of the retina, with early eruption of the tissue through the internal limiting membrane into the vitreous (Fig. 12).

Stage II.—Commencing retinal detachment, extension of fibrovascular tissue into the vitreous, and formation of fibrous strands (Fig. 13). At any time throughout Stages I and II new vessels may appear on the iris.

Stage III.—Total retinal detachment, and formation of dense retrolental fibrous membrane followed by late atrophic changes (Fig. 14).

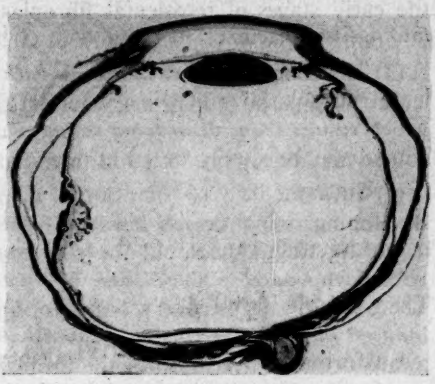

FIG. 12.—Retrolental fibroplasia. Pathological Stage I.

FIG. 13.—Retrolental fibroplasia. Pathological Stage II.

FIG. 14.—Retrolental fibroplasia: Pathological Stage III.

Summary

(1) The pathological features of four representative cases of

the early stages of retrolental fibroplasia are described in detail, and the findings are discussed in relation to cognate work.

(2) The conclusions are in accord with the widely accepted view that retrolental fibroplasia is a primary retinal disease of non-inflammatory origin, which results from disordered retinal vascularization. Its development and course may be simply stated in three pathological stages.

(3) Since the basis of the disorder is merely an aberrant overgrowth of the developing retinal vessels it is not improbable that there may be a variety of precipitating causes, but the vast majority of cases consists of premature babies who develop the disease post-natally in relation to oxygen therapy. There would thus be good grounds for applying a special name to the oxygen-induced disease, but it is suggested that a revision of nomenclature should await further clarification of the problems of pathogenesis.

My thanks are due to the clinicians and pathologists, individually acknowledged in the text, who kindly supplied me with case histories and material. I am grateful to Miss E. FitzGerald for secretarial help and to Mr. G. E. Knight and Mr. A McNeil for technical assistance.

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EFFECT OF OXYGEN ON DEVELOPING RETINAL VESSELS WITH PARTICULAR REFERENCE TO THE PROBLEM OF RETROLENTAL FIBROPLASIA

BY

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THE evidence for regarding an abnormal overgrowth of the developing retinal vessels as the pathological basis of retrolental fibroplasia has been reviewed and discussed in an earlier paper in this issue (Ashton, 1954a). This fundamental dysplasia considered alone appeared to indicate that the disease was probably no more than a violent activation of the normal process of retinal vascularization, so that the narrow confines of the nerve fibre layer could no longer contain the exuberant vaso-formative tissue, which consequently burst through the limiting membrane to invade the vitreous and so initiated a train of events terminating in total retinal detachment and the formation of a retrolental fibrous membrane. The stimulus which normally attracts the vessels into the retina is itself unknown, but it has been assumed, in general terms, to be a response to the oxygen demands of the inner layers of the retina, which the choroidal circulation is no longer able to satisfy as the eye develops. If retrolental fibroplasia began simply as an overgrowth of normal vaso-formative tissue, it seemed possible that it might occur in response to a stimulus identical in nature with the normal but excessive in degree, a variation which thus appeared to be related to a disparity between oxygen supply and demand.

On such a basis, however, it was difficult if not impossible to reconcile the apparently conflicting theories that retrolental fibroplasia was due to anoxia (Ingalls, 1948; Szewczyk, 1951, 1952; Ingalls and others, 1952; Rubinstein, 1952), or to the toxic effects of high oxygen (K. Campbell, 1951; Ryan, 1952; Goldman and Tobler, 1952; Patz and others, 1952; Von Winning, 1952), or to a relative anoxia following high oxygen administration (Crosse and Evans, 1952; Jefferson, 1952), or that oxygen was not causative or might be only one of the factors concerned (Houlton, 1951; Bembridge and others, 1952; Zacharias, 1952; Silverman and others, 1952). In an attempt to clarify the aetiological problem, it was therefore decided to carry out an experimental investigation of the influence of varying concentrations of oxygen upon the developing retinal vasculature in the kitten, which, as will be seen, is closely comparable to that in man, and a preliminary report on our findings has already been published (Ashton and others, 1953; Ashton, 1954b).

The normal development of the retinal circulation was thoroughly studied in man, the rat, and the cat by Michaelson (1948a, b), and the mode of development was found to be similar in each case. In the human embryo the

retinal foetal circulation begins to develop from the disc at the 100-mm. stage (16th week approximately), and the ingrowth gradually progresses until it reaches the periphery of the retina at the end of the 8th month; hence, in the later months of pregnancy, which particularly concern us in considering the problems of the premature infant, the vascular network is advancing between the equator and the ora serrata. In the cat embryo vascular budding commences at the disc between the 35th and 45th day of intra-uterine life, but it differs from that in man since it does not reach the periphery until about 3 weeks after birth, so that the degree of retinal vascularization in the full-term kitten at birth and in the ensuing 3 weeks is comparable in extent to that in the premature baby. Since, with minor variations, the retinal vascular development is in all other respects very similar to that in man, the kitten appeared to be the ideal animal for our purpose.

In the first few experiments it was discovered that oxygen in high concentrations was able to exert an obliterative effect on the ingrowing retinal complexes (vaso-obliterative phase) which only partially re-opened when the animal was transferred to air; retinal vascularization then recommenced from the disc region and a disordered and profuse ingrowth of vessels extended into the retina and invaded the vitreous (vaso-proliferative phase). Thus was produced a pathological process almost exactly comparable to the early stages of retrolental fibroplasia.

The purpose of the present paper is to give the experimental details of our investigation as far as space will allow, to describe the final outcome of the oxygen effect, which was unknown at the time of the preliminary communication, to report the conditions which affect the extent of the vaso-obliterative and vaso-proliferative phases of the process, and to discuss the possible mechanisms involved. Finally an attempt will be made to assess the significance of the phenomena in the aetiology of retrolental fibroplasia.

Material and Methods

121 kittens of both sexes and 41 mother or foster-mother cats were used in the preliminary study and in the 32 experiments reported here. The mothers were fed on dried milk paste in the morning, and " Kit-e-Kat " in the evening, and were given water *ad lib.* At the beginning of the investigation several animals were lost through feline virus pneumonitis, but the infection was later controlled by segregating the cats from each other and by taking aseptic precautions between cleaning and feeding each animal. Seven newborn rabbits and 91 ratlings with their mothers were also used in the investigation.

Gas Chambers.—Standard bacteriological incubators 20″ × 20″ × 24″ were converted for this purpose. The outside doors were removed and a hole was bored in the lower left corner of the inner glass door and a gas-tight metal outlet inserted. The existing thermostat hole in the top of the incubator was used for a sampling tube, and the existing thermometer hole was fitted with a gas inlet tube, both of which reached down into the lower half of the incubator. The sampling tube hung loosely through the thermometer hole so that samples could be taken from various depths within the incubator; a free escape of gas around the tube ensured that the pressure within the chamber did not arise above normal ambient pressures and this was checked with barometric readings. In the lower third of the incubator an animal sawdust tray, on which the cat and litter were placed, was supported on a shelf 4″ from the floor; beneath this shelf trays containing self-indicating soda lime (" Carbosorb ") were placed to absorb CO_2 and these were replenished 12-hrly. After the animals had been placed in the incubator and the door edges sealed with

" Sellotape ", the gas (oxygen alone or a mixture of nitrogen and oxygen) was passed through the gas inlet tube and extracted by means of an adjustable water vacuum pump from the lower outlet tube, which assisted in the removal of CO_2 that otherwise tended to accumulate in the lower part of the incubator. Temperature and hygrometer readings were taken at hourly intervals; the temperature varied between 22° and 25° C. and the relative humidity was maintained at 95 per cent.

The chambers were opened for 15–30 minutes at 12-hrly intervals for feeding, cleaning, exercise, and weighing; the gas percentages were restored to the required level in approximately 15–20 minutes and were thereafter maintained at a sufficient gas flow, which, combined with the vacuum extraction, ensured an adequate circulation of gas within the chamber. The gas flows were controlled by means of an Adams gas regulator and a rotameter graduated for oxygen.

The oxygen concentrations were checked with the BOC analyser at half-hourly intervals in the hypoxia experiments, and hourly in the hyperoxia experiments, day and night, throughout the experiments. CO_2 readings were made with a Fry's gas analyser (Fry, 1949), and at the same time the oxygen readings of the BOC analyser were checked. The CO_2 readings varied from 0–1 per cent., occasionally to 2 per cent., rising to a peak at the end of 12 hrs. The oxygen analyser was found to give accurate results within 1–2 per cent.

Controls.—In the early experiments the control kittens were reared in a control incubator in which the conditions (including the CO_2 accumulation) were identical with those in the experiment except for the oxygen concentrations, but since the retinal development was in no way affected as compared with a kitten in ordinary atmospheric conditions, subsequent controls were reared in an open cage.

Weight Gain.—A graph was made of the daily gain in weight of each animal. Kittens in hypoxia lost or failed to gain weight but there were no constant variations which could be related to the conditions of air or hyperoxia. Animals fed by a foster-mother tended to show a smaller gain in weight than those with their own mother, but this did not appear to influence the rate of retinal vascularization.

Injection Technique and Method of Examination.—At the end of each experiment the kittens were anaesthetized with 0.25 ml. Nembutal (veterinary) intra-peritoneally. The left eye was removed for histological examination and placed in 10 per cent. Formol saline. The thoracic cavity was then opened and the condition of the organs noted; the descending aorta was ligatured and the pericardium incised; a hypodermic needle, connected with polythene tubing to a 55-ml. syringe containing Indian ink, was then inserted into the left ventricle, and, after incising the right auricle, 25 ml. ink were gently injected. The right eye was then removed and placed in 10 per cent. Formol saline for 12 hrs and subsequently frozen in CO_2 snow and opened transversely by an incision through the ora serrata. The specimen was then examined under a dissecting microscope with direct illumination. In the anterior portion the condition of the iris and tunica vasculosa lentis was recorded. In the posterior portion the degree of retinal vascularization and the condition of the hyaloid artery were studied *in situ*. The retina was then removed *in toto*, cut radially, mounted flat, and photographed. The maximum outgrowth of vessels from the disc was measured with dividers and recorded.

Histology.—Celloidin sections of the uninjected eye were cut and stained with haematoxylin and eosin, and periodic acid-Schiff stain.

Preliminary Study.—As a preliminary study the injected retinae of normal kittens were examined at the following ages:

at birth, 6 and 12 hrs, 2, 3, 5, 6, 9, 11, and 17 days, and 5 and 8 weeks.

The appearances and mode of development were found to be as Michaelson (1948a, b) had described. The upper temporal complex always gave the maximum outgrowth readings, but the nasal complex was always nearer to the ora. The variation in the extent of vascularization between animals of the same age from different litters was found to be considerable, as Michaelson had also reported, so that it was not satisfactory to compare the test animal with a normal animal of the same age unless they were from the same litter and of comparable weight. In our later experiments, therefore, the experimental kitten was compared with a litter mate of the same age reared in air with a foster mother.

Experimental Findings

The records of our experiments are unusually complicated and this is partly due to the fact that unexpected deaths among the animals meant that the actual data provided was not always exactly that which had been sought, so that to give an accurate account of the investigation it would be necessary to include both the experimental plan and result as shown in our preliminary report. Space does not, however, permit inclusion of our full experimental protocol, but the essential details

are presented in Table I (*see* pp. 402–403), and Tables II, III, and IV (*see* Appendix, pp. 430). The histological findings will be described in the text.

Since the conditions of each experiment were influenced by factors beyond our control, such as the varying age of available kittens and the illness or death of animals during an experiment, it is impossible to divide all the experiments into groups which are exactly similar in every detail. It is, therefore, necessary to adopt standard criteria which may be used to classify the results into approximately comparable groups, and the following standards have been employed for this purpose.

(*i*) Concentrations of Oxygen (per cent.) 70–80
45–55
35–45
25–35

(*ii*) Periods of Exposure (days) 3–7
8–14
15–21
22 and over

(*iii*) Age Groups according to Age at First Day of Oxygen Exposure (days) 1–7
8–14
15–21
22 and over

(*iv*) Extent of Vaso-Obliterative Effect

Total++++	Severe+++	Moderate++
Mild+	Doubtful±	No effect 0.

We shall now proceed to analyse the findings, with reference to the appropriate experimental numbers, under the following headings:

(1) Vaso-obliterative effect of hyperoxia.

(2) Factors influencing vaso-obliterative effect:
- (*a*) Degree of maturity of retinal vessels;
- (*b*) Duration of exposure to hyperoxia;
- (*c*) Concentration of oxygen.

(3) Effect of transfer to air after exposure to hyperoxia:
- (*a*) Fate of obliterated vessels;
- (*b*) Vaso-proliferation.

(4) Factors influencing obliterated vessels:
- (*a*) Prolonged hyperoxia;
- (*b*) Hypoxia;
- (*c*) Concentration of carbon dioxide;
- (*d*) Priscol;
- (*e*) Tromexan;

(5) Factors influencing vaso-proliferation;
- (*a*) Duration of exposure to oxygen;
- (*b*) Effect of re-oxygenation.

(6) Effect of hypoxia.

(1) VASO-OBLITERATIVE EFFECT OF HYPEROXIA

High concentrations of oxygen at atmospheric pressure are able to obliterate the ingrowing vessels in the developing retina of the kitten; in some circumstances this effect is complete (Figs 1 and 2) and in others partial. Such results have been obtained repeatedly (complete twelve times in experiments 1, 4, 7, 20, 23, and 24; partial sixteen times in experiments 2, 10, 11, 16, 21, 25, 27, 31, and 36), and it has

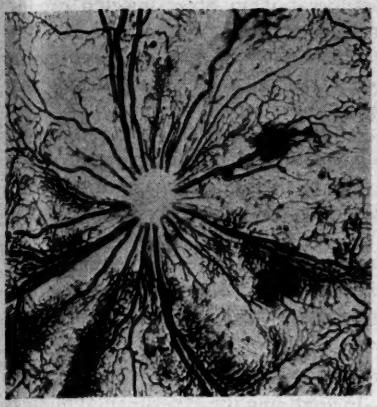

FIG. 1.—Control kitten, 1 day old (Exp. 4, k1), showing disc region of normal developing retina. Note density of vascular complexes. Injected Indian ink. Mounted flat. ×14. (Whole retina shown in preliminary report).

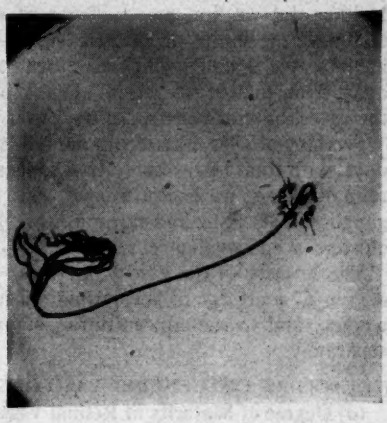

FIG. 2.—Experimental kitten, 9 days old (Exp. 1, k3), showing effect of 6 days' continuous hyperoxia (70–80 per cent. O_2). Apart from a few attenuated vessels around disc, retinal vasculature has been totally obliterated. Hyaloid artery and tunica vasculosa lentis not affected. Injected Indian ink. Mounted flat. ×11. (Cf. Fig. 1).

been found that, given the same experimental conditions, very similar results may be reproduced with remarkable regularity. The same process, although to a much less marked extent, may also be demonstrated in the developing retinal vessels in the immature eye of the young rabbit and ratling. In the rat, however, the peripheral vessels are little affected, vaso-obliteration being mainly in the post-equatorial region.

Since this phenomenon was reported, its development has been studied by direct observation of the living kitten retina, using a limbal window technique, and the findings are described in a preliminary report (Ashton and Cook, 1954); here we shall describe only those features which are revealed by injection methods.

Study of retinae in which vaso-obliteration was not fully developed showed that the process commenced as a marked constriction of the arteries and arterioles; the arterioles and the arterial side of the capillary circulation were the first vessels to obliterate, and this process was followed by the disappearance of the whole capillary bed; then the main arteries and finally the main veins closed, leaving an apparently avascular retina. Usually the blood was squeezed out of the vessels as they collapsed, but blood clot might be trapped within isolated areas. In general the process began peripherally, that is in the region where the vessels were most immature, and spread posteriorly, so that the disc region appeared to be the most resistant and was the last area to be completely obliterated.

It is of interest to note, however, that the capillary bed lying between the closely related arteries at the disc frequently disappeared early. In cases where obliteration was incomplete, the persisting arteries and veins around the disc might be united through peripheral arterio-venous anastomoses (Fig. 23).

The other vessels in the eye, such as the tunica vasculosa lentis, hyaloid artery, and choroidal circulation, showed no abnormality in the injected specimens.

Neither did we find any evidence of vaso-obliteration in other injected organs, including the brain, of animals showing total closure of the retinal vessels. Nor have treated animals which have been allowed to survive, shown any abnormality apart from that of the retina. Our experiments thus led us to the conclusion that the vaso-obliterative effect of oxygen is confined to the retinal vessels.

Sections of eyes taken from animals subjected to 70–80 per cent. oxygen for 3–7 days, with total vaso-obliteration as seen in the fellow injected eye, showed no patent retinal vessels, no haemorrhages, and no evidence of retinal degeneration. The ganglion cells appeared normal, and the closed vessels were represented by small clusters of endothelial cells.

The severity of the vaso-obliterative effect was found to be proportional to the degree of maturity of the retinal vascularization, to the duration of exposure to oxygen, and to the concentration of oxygen; these factors will now be discussed separately.

(2) FACTORS INFLUENCING VASO-OBLITERATIVE EFFECT

(*a*) **Degree of Maturity of Retinal Vessels.**—In analysing the experimental findings to determine the importance of the degree of maturity of the retinal vessels as a factor in the vaso-obliterative effect, the standard experimental conditions adopted were those known to be capable of producing total vaso-obliteration, *i.e.* 70–80 per cent. oxygen for 3–7 days. Table I shows the extent of obliteration when the four age groups were subjected to this standard exposure.

TABLE I

ANALYSIS OF 21 EXPERIMENTS (45 ANIMALS) INVOLVING A SIMPLE EXPOSURE TO OXYGEN

Showing extent of vaso-obliteration in four age groups following exposure to varying concentrations of oxygen for four periods of time

Oxygen Exposure (days)	Age Group (days)	Oxygen (per cent.)	Experiment Number	Age (days)	Exposure (days)	Vaso-obliteration
3–7	1–7 and 8–14	70–80	1, k3	3	6	++++
			4, k4	1	4	++++
			2, k2	2	3	+++
			1, k1	3	4	++++
			1, k2	3	3	++++
			20, k3	8	3	++++
			7, k5	8	5	++++
			27, k1	12	4	+++
			27, k2	12	4	+++
			31, k2	12	5	+++
		45–55	21, k1	8	4	+++
		35–45	16, k1	4	4	+++
			16, k2	4	3	+++
			16, k3	4	4	+++
			16, k5	4	4	+++
			10, k1	3	4	+++
			10, k2	3	4	++
		25–35	15, k1	1	7	±
			29, k1	3	5	0
			29, k2	3	7	0
			29, k3	3	7	No result

TABLE I—*continued*

Oxygen Exposure (days)	Age Group (days)	Oxygen (per cent.)	Experiment Number	Age (days)	Exposure (days)	Vaso-obliteration
3–7	15–21	70–80	11, k1	17	4	+
	22 and over	70–80	13, k1	29	7	±
			14, c2	50	7	0
			8, k1	58	5	0
			23, c	Adult	9	0
			24, c	Adult	9	0
8–14	1–7 and 8–14	70–80	24, k3	4	9	++++
			24, k2	4	13	++++
		25–35	15, k4	1	13	0
	15–21	70–80	25, k1	18	10	++
			25, k2	18	10	++
			36, k1	18	10	+
			36, k4	18	13	+ (died)
	22 and over	70–80	13, k2	29	12	0
15–21	1–7 and 8–14	70–80	24, k1	4	15	No result
			23, k1	3	20	++++
			23, k2	3	16	++++
			23, k3	3	21	++++
		25–35	35, k1	4	21	0
			35, k2	4	20	No result
			35, k3	4	21	0
	15–21	70–80	36, k5	18	20	0
	22 and over	70–80	14, c1	50	16	0
22 and over	1–7 and 8–14	70–80	23, k4	3	26	++++

Obliteration was total or severe at 1–14 days, mild at 15–21 days, and doubtful or absent at 22 days and over; that is, during the earlier periods of vascular growth, the vessels are highly susceptible to oxygen, becoming gradually less so until the final period of retinal vascularization is reached, when only a mild obliterative effect occurs. Once the retinal circulation is fully established and the normal vascular architecture attained, that is at about 21 days old, oxygen fails to produce significant obliterative changes, and it has no structural effect whatsoever on the vessels of the adult cat.

It is thus apparent that the severity of the vaso-obliterative effect of oxygen is inversely proportional to the maturity of the retinal vessels, and this conclusion is in keeping with our previous observation that the obliterative process begins peripherally and spreads posteriorly, the main vessels of the disc region being the least susceptible.

(*b*) **Duration of Exposure to Hyperoxia.**—The influence of the period of exposure to oxygen can be deduced to a limited extent from the experiments. In Experiment

32, for instance, three kittens of 2 days old were subjected to 70–80 per cent. oxygen for 12, 24, and 36 hrs respectively and were then killed immediately. Vaso-obliteration was moderate in the first animal, severe in the second, and total in the third, thus showing that the vaso-obliterative effect is dependent upon the period of exposure, begins in less than 12 hrs, and is complete by 36 hrs (Figs 3, 4, and 5).

FIG. 3.—Exp. 32, k1, after 12 hrs' exposure to 70–80 per cent. O_2. Vaso-obliteration moderate. Whole retina. ×4.

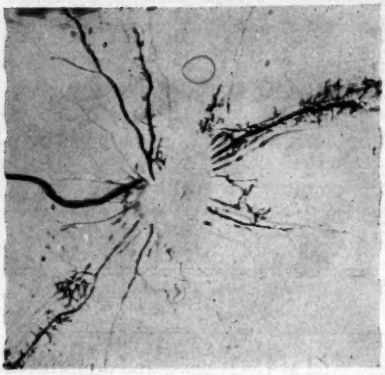

FIG. 4.—Exp. 32, k2, after 24 hrs' exposure to 70–80 per cent. O_2. Vaso-obliteration severe. A few capillaries and main vessels persist posteriorly. Disc region. ×12.

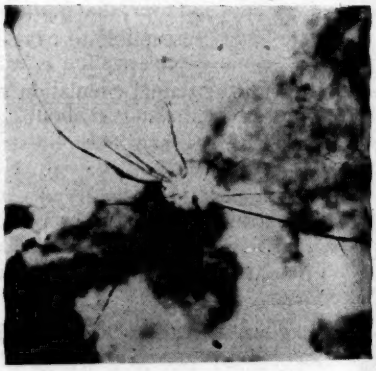

FIG. 5.—Exp. 32, k3, after 36 hrs' exposure to 70–80 per cent. O_2. Vaso-obliteration total. Only a few attenuated vessels remain around disc. Disc region. ×12.

Longer periods of exposure to oxygen concentrations are analysed in Table I.

70–80 per cent. oxygen.—In the most sensitive age groups (1–14 days) the total obliteration produced in 3–7 days continued unchanged through the longer periods up to the longest time studied (26 days, Exp. 23 k4). In the less sensitive group (15–21 days old) vaso-obliteration was partial and peripheral; the mild effect (+) obtained in Exp. 11, k1, increased to a moderate effect (++) in Exp. 25, k1, k2, when the period of exposure was more than doubled; on the other hand an animal of the same age group (Exp. 36, k5) showed no vaso-obliteration after 20 days' exposure to 70–80 per cent. oxygen, so that there is obviously some individual variation in these experiments. In the insensitive age group (22 days old and over) these concentrations of oxygen had no effect, irrespective of the period of exposure (up to 16 days).

35–55 per cent. oxygen.—Not investigated at varying periods.

25–35 per cent. oxygen.—These concentrations had no vaso-obliterative effect on the most sensitive age group (1–14 days) irrespective of the period of exposure (up to 21 days).

These long-term experiments show, therefore, that total vaso-obliteration once induced remains unaltered for at least 26 days' continuous exposure, and that retinal vessels remain unaffected by oxygen concentrations below 35 per cent. for at least 21 days' continuous exposure. The conversion of a mild to a moderate degree of vaso-obliteration in the 15–21 age group by increasing the exposure period (Exp. 11, k1 and 25, k1, k2) was not confirmed in repeat experiments (36, k1, k2).

In conclusion, it is apparent that the experiments investigating short periods of oxygen exposure (12, 24, and 36 hrs) show that the vaso-obliterative effect is directly related to the time of exposure, but this relationship is not conclusively shown in the studies of longer periods.

(*c*) **Concentration of Oxygen.**—The importance of oxygen concentrations as a factor in the vaso-obliterative effect is shown in Table I. It has already been shown that the most sensitive age groups were those between 1–14 days old, and Table I shows that in these age groups, in a standard exposure time (3–7 days), vaso-obliteration is complete (++++) with 70–80 per cent. oxygen, severe (+++) with 35–55 per cent. oxygen, and doubtful (±) or absent (0) with 25–35 per cent. oxygen.

In animals of the older age groups (15 days and over), the highest concentration of oxygen (70–80 per cent.) produces only a mild vaso-obliteration in the 15 to 21-day-old group and a doubtful effect or none in the group known to be insensitive (22 days old and over): it was, therefore, presumed that lower concentrations would have even less effect and no further experiments were carried out on this age group.

It is thus apparent that the severity of the vaso-obliterative effect of oxygen is directly proportional to the concentration of oxygen and that concentrations below 35 per cent. have little or no effect in the most sensitive age groups.

(3) EFFECT OF TRANSFER TO AIR AFTER EXPOSURE TO HYPEROXIA

(*a*) **Fate of Obliterated Vessels.**—When an animal which has been subjected to sufficient concentrations of oxygen to induce total retinal vaso-obliteration is transferred to air, the obliteration is found to be irreversible in the majority of the affected vessels. Such vessels as remain patent, or are able to re-open, refill with blood, but the resulting vascular architecture is grossly abnormal and consists of an attenuated, irregular cobweb of vessels without definition into arteries or veins; haemorrhages

may appear throughout, or at the periphery of, the re-opening network (Figs 6 and 7). In the living animal it is possible that this degree of re-opening is even less than it appears to be in the *post-mortem* specimens, for the injection of Indian ink may to some extent force the vessel walls apart.

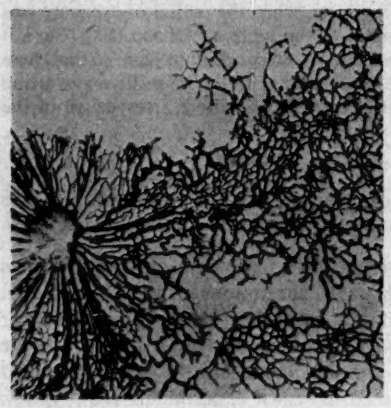

FIG. 6.—Exp. 4, k6, showing effect of 4 days' exposure to 70–80 per cent. O_2 followed by 3 days in air. The pre-existing vascular complexes have partially re-opened but the capillary network is of a grossly abnormal architecture, being cobweb in type and without definition into arteries and veins. Vascularization of retina is recommencing from disc region. ×14. (Whole retina shown in preliminary report).

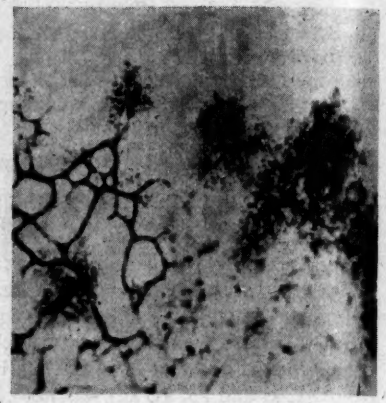

FIG. 7.—Periphery of re-opened network shown in Fig. 6. Re-opened vessels tail off into thrombosed capillaries from which many haemorrhages have occurred. ×50.

We believe that the closure of the vessels is rendered permanent either by thrombosis or by the adherence of the apposing endothelial cells through plasma coagulation; the latter process is probably the more important, for no residual blood is present in the majority of collapsed vessels (this point will be further discussed in Section 4). It would, therefore, appear that the reversibility of vaso-obliteration depends upon the duration of exposure to oxygen, but in this series of experiments we have not determined the maximum period of vaso-obliteration which is completely reversible. This can only be satisfactorily assessed by direct observation of the retinal vessels in the living animal: further work on this aspect of the problem is reported in a subsequent paper (Ashton and Cook, 1954).

It is clear, however, from our injection experiments, that, where total obliteration was obtained by oxygen exposure for 3 days or more, the vascular architecture was never restored to normal on transfer to air.

In experiments where total vaso-obliteration was produced, the attenuated cobweb of vessels resulting from partial re-opening in air appeared to be inadequate to nourish the retina, and it gradually atrophied and disappeared as vascularization recommenced from the disc region, so that the final revascularized retina contained none of the original retinal vessels. In our experiments, where only partial obliteration was produced, it is difficult to decide if re-opened vessels persisted or not, but the survival of such vessels probably depends upon whether the degree of re-opening permits an adequate blood flow to nourish the cells of the vessel walls, and it is likely that much of the re-opened network persists in cases where only partial vaso-obliteration occurs.

As regards histological appearances, it has already been stated that the closed vessels are represented by clusters of endothelial cells which may give the appearance of increased cellularity in the inner layers of the retina; if, on transfer to air, the channels do not permanently re-open, the cells atrophy and disappear without trace in the finally revascularized retina. Nor does the new-vessel ingrowth show any tendency to grow along the same pathways as those of the atrophied vessels.

(*b*) **Vaso-Proliferation.**—The features of the vaso-proliferative phase in air differ according to whether the vaso-obliterative effect is complete or partial. Where the *maximum* effect is produced in oxygen, then, as stated above, the re-opened network is attenuated and gradually disappears; meanwhile revascularization of the retina recommences from the disc region and is well established as early as 3 days after transfer to air (Exp. 4, k6), as is shown in Fig. 6. Even at this stage it is seen that the ingrowth is abnormal in character, containing a greater number of vessels and forming a richer plexus than normal. By the 9th day (Exp. 7, k2) there is a profuse circular outgrowth of vessels, several layers deep, without definition into the normal three complexes, and the fine cobweb of re-opened vessels has disappeared. By the 18th day (Exp. 7, k6 and Exp. 30, k1) the vascular proliferation is even more luxuriant, and on the surface of the outgrowth there are numerous corkscrew proliferations which extend into the vitreous as very fine arborizations or as small glomerular tufts. Numerous uncanalized and partially canalized endothelial buds may be demonstrated in the vitreous by staining with toluidine blue (Figs 8, 9, 10, 11, and 12, overleaf). At the same time there is a suggestion of new vessel formation on the anterior surface of the iris. Histologically there is evidence of intense angioblastic activity in the inner layers of the retina with extension of vaso-formative tissue and vessels into the vitreous, but there is no apparent degenerative change in the retinal neural elements and no retinal detachment (Fig. 13, overleaf). By the 30th day (Exp. 7, k3) the revascularization has progressed in a disorderly fashion in several layers within 2–3 mm. of the ora serrata in all directions; over the whole surface of the corrugated revascularized retina there is a dense network of new vessels growing into the vitreous as loops and glomerular tufts. The ingrowing vessels have now differentiated into arteries and veins. Sections confirm these findings, but now show that additional feature of multiple, acute-angled folding in the outer layers of the retina (Fig. 21), an appearance which suggests contraction of the neovascular tissue in the retina or a disproportionate development between the outer and inner layers. No true detachment is present.

The *long-term results* of the total vaso-obliterative effect of oxygen are demonstrated in Exp. 12. After about 2¼ months (k3, k5) the retina is extensively revascularized, and posteriorly the vessels have completely differentiated into arteries and veins of which there are more than normal; the profuse intravitreal proliferation is now disappearing in this area but many long convoluted vascular loops remain. The periphery of the ingrowth at the equatorial region, however, shows a wide band of dense and multi-layered vessels; many vessels extend into the vitreous as loops and glomerular tufts, and the picture is exactly like that which existed in the disc region about 7 weeks previously (Figs 14, 15, 16, and 17, overleaf).

After about 5¼ months (k6) the retina is almost entirely revascularized, the vessels are differentiated into arteries and veins, and there are now only two main capillary networks, but the general architecture differs considerably from the normal, there being as many as twenty main vessels issuing from the disc region (Figs 18, 19, and 20, overleaf). Many long vascular loops are seen extending into the vitreous,

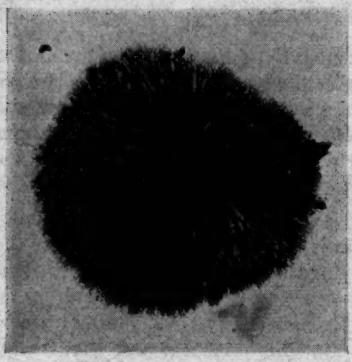

FIG. 8.—Exp. 7, k6, showing effect of 5 days' 70–80 per cent. O_2 followed by 18 days in air. The re-opened network has now disappeared and revascularization is advancing from the disc, where there is a profuse circular ingrowth of the vessels several layers in depth. The pattern of growth is completely abnormal and on the surface of the ingrowth there are corkscrew proliferations extending into the vitreous as fine arborizations or glomerular tufts. ×5.

FIG. 9.—High-power view of Fig. 8, showing vascular branches extending into vitreous. ×22.

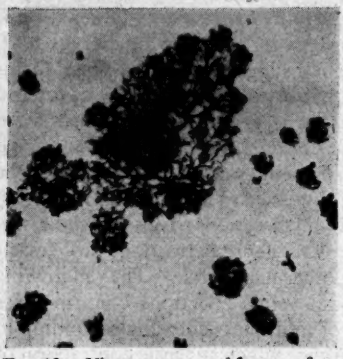

FIG. 10.—Vitreous removed from surface of vascular ingrowth shown in Fig. 8. Numerous injected glomerular tufts are present. ×22.

FIG. 11.—High-power view of glomerular tuft shown in Fig. 10; it consists of a canalized mass of endothelial cells. ×320. (Cf. Figs 3 and 4, Ashton, 1954a).

FIG. 12.—Uncanalized masses of endothelial cells from vitreous of case shown in Fig. 8. Toluidine blue. ×426.

FIG. 13.—Section of retina from case shown in Fig. 8. Vaso-formative tissue extends from inner layer of retina into vitreous. Haematoxylin and eosin. ×340. (Cf. Fig. 10, Ashton, 1954a).

FIG. 14.—Exp. 12, k5, showing progress of retinal re-vascularization after about 2 months in air. Posteriorly (below) vessels have differentiated into arteries and veins of which there are more than normal. Intravitreal proliferations are disappearing in this area but many loops remain (Fig. 16). At the periphery (above) there is a wide band of multi-layered vessels with numerous intravitreal loops and glomerular tufts (*See* Fig.15). ×8.

FIG. 15.—Exp. 12, k5, high-power view of periphery of ingrowth showing multi-layered vascular network and intra-vitreal glomerular tufts. ×24.

FIG. 16.—Exp. 12, k5, high-power view of posterior ingrowth showing long convoluted loops extending into vitreous. ×24.

FIG. 17.—Exp. 10, k6, delicate network of vessels extending into vitreous from inner layer of retina in which there is marked angioblastic activity. Haematoxylin and eosin. ×88. (Cf. Fig. 5, Ashton, 1954a).

FIG. 18.—Showing progress of revascularization after about 5 months in air. The vessels have completely differentiated into arteries and veins and there are now only two main capillary networks. Many long vascular loops extend into the vitreous equatorially and numerous dot-like glomerular tufts may be seen at the periphery (above). The vascular pattern is grossly abnormal and there are many more large vessels around the disc than normal. ×5.

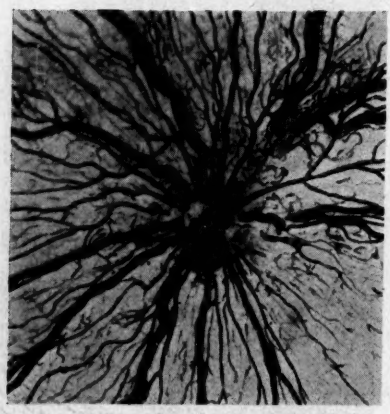

FIG. 19.—Disc region of revascularized retina. Note large number of main vessels. ×14. (Compare with normal in Fig. 20).

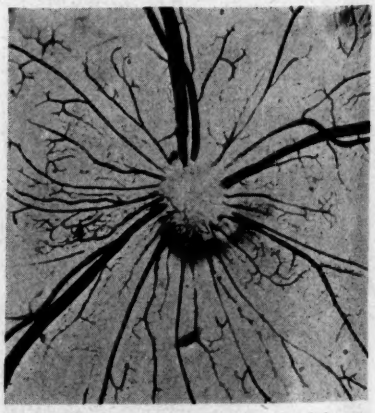

FIG. 20.—Disc region of normal adult cat. ×14. (Compare Fig. 19).

particularly equatorially, while at the periphery the loops are shorter and more numerous, and there are several glomerular vascular tufts. There is no histological evidence of excessive angioblastic activity in the inner layers of the retina, but vessels may be seen in the vitreous and there are small quantities of structureless eosinophilic material on the retinal surface, probably representing atrophied vascular tissue.

Apart from these abnormalities, the histological picture of the retina has been restored to normal, and the only definite evidence of its previous disruption is the presence of numerous acute-angled folds in the posterior half of the eye, some of which give a rosette appearance (Figs 21 and 22); there is no evidence of retinal detachment.

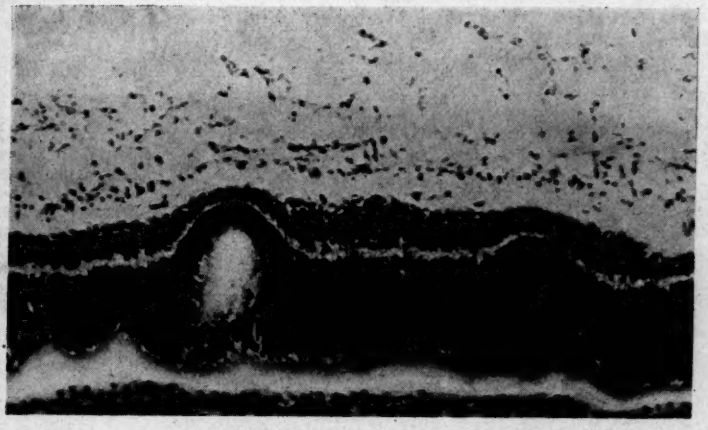

FIG. 21.—Exp. 27, k3, section of retina after 21 days in air following 4 days in 70–80 per cent. O_2. The nerve fibre layer is thickened by increased quantities of vasoformative tissue and numerous vessels extend into the vitreous. Multiple acute-angled folds are seen in the outer layers of the retina, but there is no true detachment. Haematoxylin and eosin. ×120.

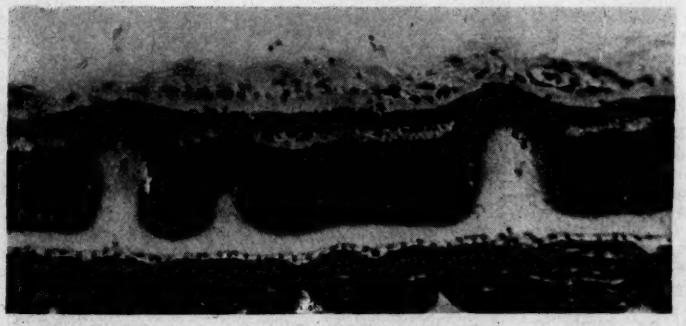

FIG. 22.—Exp. 12, k5, section of retina after 67 days in air following 4 days in 70–80 per cent. O_2. There is now no angioblastic activity in the nerve fibre layer and the intravitreal vessels have atrophied. Irregular eroded areas are present on the retinal surface and acute-angled folds persist in its outer layers. Haematoxylin and eosin. ×120.

One animal of this experiment (Exp. 12, k4), in which a maximum vaso-obliterative effect was induced, is still alive and has now been observed for 10 months. The vaso-proliferative phase was studied during development and regression; retinal detachment did not occur and excellent vision appears to be retained. The retinal vascular pattern, however, is grossly abnormal, with many more large vessels around the disc than normal. In all other respects the animal is healthy.

When the vaso-obliterative effect of oxygen is *partial*, vascularization of the retina does not recommence from the disc region as in cases where total obliteration is produced. In Exp. 27, for instance, the vaso-obliteration was extensive but not complete since the kittens were of a less sensitive age group (12 days); three vestigial complexes persisted with peripheral arterio-venous anastomoses (Fig. 23). After 21

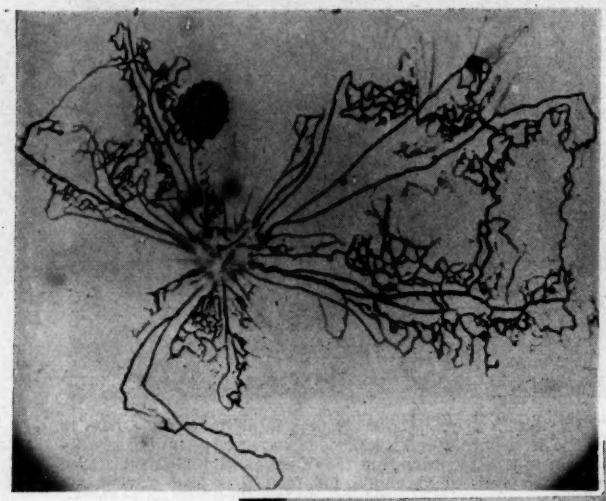

FIG. 23.—Exp. 27, k1, partial but severe vaso-obliteration induced by exposure to 70–80 per cent. O_2 for 4 days. Note development of arteriovenous anastomoses at periphery of partially obliterated complexes. ×15.

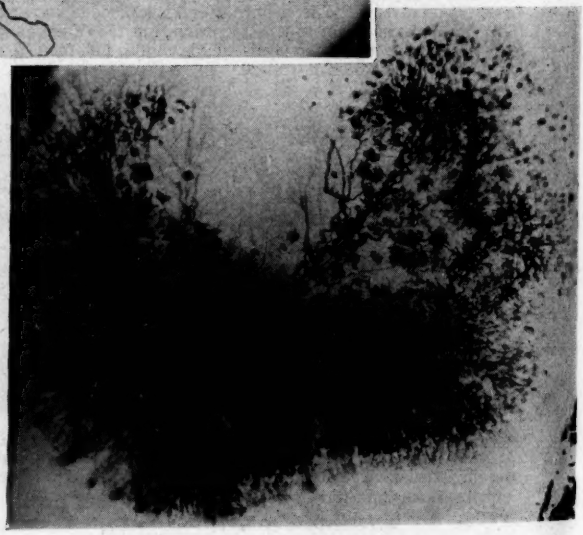

FIG. 24.—Exp. 27, k3, showing effect of 21 days' survival in air after the partial obliteration illustrated in Fig. 23. Revascularization from disc has not taken place but profuse vaso-proliferation has occurred over the whole field of unobliterated vessels, and from such vessels as have re-opened. Numerous loops and glomerular vessels are present in the vitreous on the surface of the retina. ×7.

days in air (k3), there was a massive disorderly vaso-proliferation with glomerular and looped vessels invading the vitreous, but the new-formed vessels originated from the whole field of unobliterated vessels and from such vessels as had re-opened. Although the disc region shared in this general proliferation, vascularization of the retina did not recommence from this site as in cases in which total obliteration had been induced (Fig. 24). Otherwise, however, the process was exactly similar.

Exp. 36 is particularly interesting, for it shows that when partial vaso-obliteration is restricted to the periphery or anterior portion of the retina (k1), then the subsequent vaso-proliferation is similarly confined and no proliferations occur posteriorly (k2, Fig. 25). This experiment further shows the marked variability in response to oxygen exposure at this age; two other animals of the litter (k5, k3) showed no vaso-obliteration or vaso-proliferation although exposed to 70–80 per cent. oxygen for the same or longer periods.

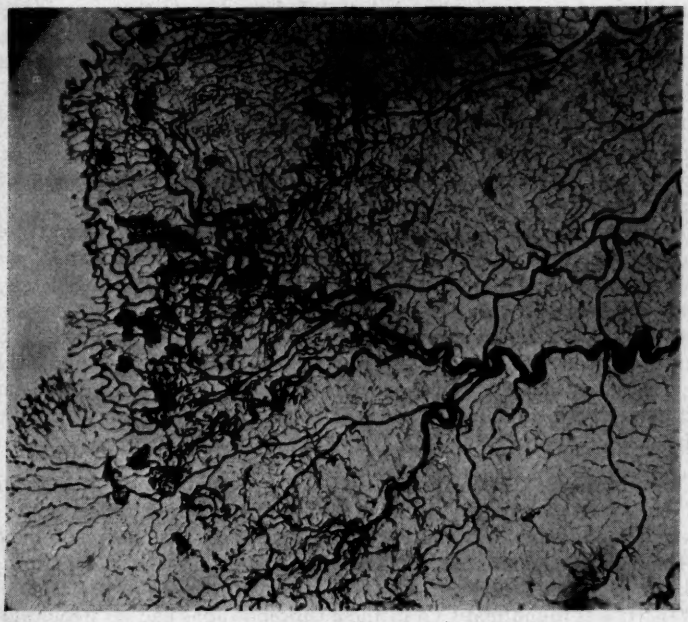

FIG. 25.—Exp. 36, k2. In this 18-day-old animal vaso-obliteration occurred only at the periphery after 10 days' exposure to 70–80 per cent. O_2. Note that after 18 days' survival in air vaso-proliferation, consisting of tufts and loops, is similarly confined to the periphery (left). Revascularization of the retina did not take place and no vaso-proliferations occurred posteriorly. This is the type of vaso-proliferation that occurs in most cases of retrolental fibroplasia. ×19.

The rate of growth of the vessels in retinal revascularization after total obliteration is much slower than normal, particularly in the terminal stages. No accurate figures are, of course, available, and in any event, there is considerable variation between animals, but in general it may be said that the normal retina is completely vascularized in about 5–6 weeks (*i.e.* 18 days ante-natal and 21 days post-natal), whereas the abnormal ingrowth may not be complete for 5–6 months.

That this delay occurs in the later stages of revascularization is shown by the following comparison of approximate times:

Vascularization	At Equator	Reaches Ora	Complete
Normal	3 wks	5 wks	6 wks
Abnormal	5 wks	10 wks	22 wks

No rats survived after oxygen exposure, so that we were unable to study vaso-proliferation in these animals. Vaso-proliferation did not occur in rabbits, because the partially obliterated vessels re-opened adequately on transfer to air.

(4) FACTORS INFLUENCING OBLITERATED VESSELS

(*a*) **Prolonged Hyperoxia.**—Experiments in which exposure to hyperoxia was continued beyond the standard period of 3–7 days showed that, providing there were no pulmonary complications, the obliterated vessels remained closed as long as the animal was in oxygen; thus in Exp. 23 the vessels remained obliterated for 16, 20, and 21 days, and in Exp. 24 for 9, 13, and 15 days.

(*b*) **Hypoxia.**—It would be of interest to know whether a transfer to hypoxia rather than to air has any influence upon the degree of re-opening of vessels or upon the subsequent vaso-proliferative phase. At the present time, however, only one experiment of this kind has been carried out (Exp. 4).

Total oxygen vaso-obliteration was induced in these animals by 4 days' 70–80 per cent. oxygen, but one animal (k5) was transferred to hypoxia (10–15 per cent. oxygen) for 3 days, and a control (k6) to air for the same period. There was no significant difference between the degree of re-opening, neither was there a greater ingrowth of vessels in the hypoxic animal, as might have been expected. It is possible that animals kept for a longer period in hypoxia and air might show a difference, but, in view of our inability to influence normal retinal vascularization to any very marked degree with prolonged hypoxia (cf. under Section 6), this would appear unlikely.

(*c*) **Concentration of Carbon Dioxide.**—In order to eliminate the accumulation of CO_2 in the oxygen incubators as a factor in the vaso-obliterative effect and to determine whether a possible vaso dilating action of a raised CO_2 tension on the retinal arterioles might antagonize the obliterative action of oxygen, an experiment was carried out in which 5 per cent. CO_2 was added to the 60–70 per cent. oxygen (Exp. 17). It was found that CO_2 in these concentrations had no significant effect upon the phase of vaso-obliteration, and re-opening of vessels and vaso-proliferation occurred as in the control animals.

(*d*) **Priscol** (tolazoline hydrochloride).—In order to determine whether vaso-obliteration might be antagonized by a vaso dilator, an animal was treated for 48 hrs before and during oxygen exposure with Priscol (Exp. 31), which is known to have a vaso dilating action on retinal vessels in man. The test animal (k1) was given 12.5 mg. Priscol orally 6 hrly, which proved to be the maximum dose the animal could tolerate. After 5 days' exposure to 70–80 per cent. oxygen the test and control animals were killed. There was extensive vaso-obliteration in both animals, with no significant difference between the two. It may thus be concluded

that in this single experiment Priscol did not appear to influence the vaso-obliterative effect of oxygen.

(e) **Tromexan** (ethyl biscoumacetate).—Since we believe that the closure of vessels by hyperoxia is rendered permanent by thrombosis or adherence of the vascular walls through plasma coagulation, it was thought that anticoagulants might permit re-opening of the vessels when the animal was transferred to air. Four experiments to investigate this point were therefore carried out (Exps 18, 20, 21, and 22).

In these experiments Tromexan was administered as follows: Initial dose 100 mg./kg. body weight, suspended in 1 ml. methyl cellulose, per stomach tube 24 hrs before oxygen exposure. Subsequent dosage was adjusted to maintain the prothrombin level at 3 to 4 times the normal; prothombin time was estimated by Quick's micro-technique on blood obtained from the pads. In practice it was difficult to maintain the prothrombin time constantly at the planned level.

(i) In the first experiment (Exp. 18) the animal pre-treated with Tromexan showed less avascular areas in the retina after 2 days in air following oxygen exposure than the control animal, thus suggesting that Tromexan could facilitate re-opening of the vessels, but the prothrombin times fluctuated over wide limits so that the experiment was not conclusive.

(ii) The second experiment (Exp. 20), however, was more informative and showed a very definite difference between the control and test animals, there being a total obliteration in the untreated animal (++++) and only a partial obliteration (++) in the kittens receiving Tromexan. Unfortunately the animals died before they could be transferred to air for sufficient time to observe fully the difference in the re-opening of the complexes.

(iii) The third experiment (Exp. 21) again showed that Tromexan affords a considerable degree of protection from the obliterative effect of hyperoxia (Figs 26 and 27, overleaf), but, contrary to expectations, the Tromexan-treated animals maintained in air for 3 days after oxygen exposure showed no marked difference from the Tromexan-treated animal killed within minutes of removal from oxygen.

(iv) The fourth experiment (Exp. 22) gave anomalous results and no conclusions were possible.

These experiments, therefore, show that Tromexan opposes, at some stage, the obliterative action of oxygen, and that this antagonism is evident immediately the animal is returned to air. If we assume that the drug achieves this effect by virtue of its anticoagulant properties, then, since there is no reason to suppose that an anticoagulant *per se* could interfere with the obliterative action of oxygen, the results would indicate that thrombosis or plasma coagulation is prevented within the closed vessels, thus permitting them to re-open on transfer to air. The fact that this effect is evident a few minutes after removal from oxygen suggests that the vascular closure itself is due to spasm, which, providing intravascular coagulation is prevented, is rapidly reversible and may be overcome by the death of the animal or possibly by the force of the Indian ink injection.

If this is the explanation of our findings in these Tromexan experiments, it is clear that all our injection preparations merely demonstrate the extent of vascular occlusion and do not show the actual degree of vascular closure due to oxygen which may have been present in life. On the other hand, Tromexan may have other unknown properties, such as a vasodilator action, which may directly oppose the action of oxygen, but the experiment with Priscol would appear to elimniate this possibility. Clarification of these points must await further experiment, particularly by direct observation of the effect of oxygen on the retinal vessels in living animals both treated with Tromexan and untreated.

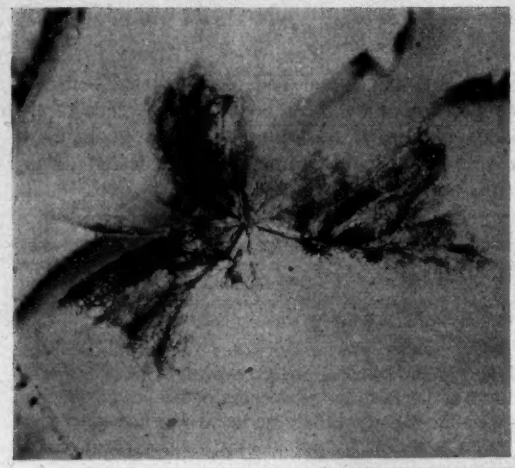

FIG. 26.—Exp. 21, k1 (Tromexan experiment, control animal), showing severe degree of vaso-obliteration after 4 days' exposure to 45–55 per cent. O_2. ×4. (Compare Fig. 27).

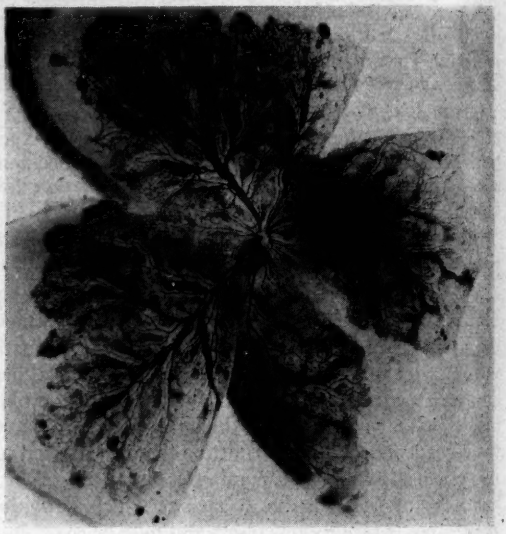

FIG. 27.—Exp. 21, k5 (Tromexan experiment, test animal), showing almost normal vascular complexes. Vaso-obliteration is very slight and considerably less than in the control. ×4. (Compare Fig. 26.)

(5) FACTORS INFLUENCING VASO-PROLIFERATION

(*a*) **Duration of Exposure to Oxygen.**—With regard to the relationship of vaso-proliferation to the period of exposure to oxygen, which we have already shown is an important factor in the vaso-obliterative effect, there are two important questions to consider:

(*i*) *Is the degree of vaso-proliferation in air related to the duration of hyperoxia?*

Doubtful information in answer to this question is provided by several of our experiments (7, k3; 10, k6; 10, k5; 12, k2), but the only experiments which are strictly comparable for this purpose are Exp. 7 and Exp. 28. One kitten of the former (Exp. 7, k6) was 8 days

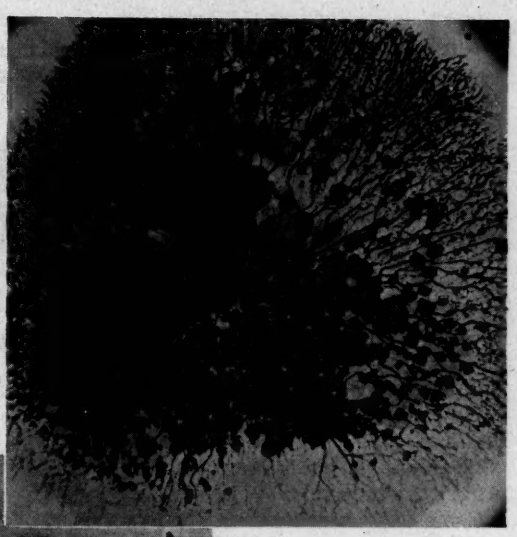

FIG. 28.—Exp. 28, k1, showing effect of 18 days' survival in air after exposure to 70–80 per cent. O_2 for 10 days. The retina is revascularizing and there is profuse intravitreal extension of vascular tufts and loops. Intravitreal growth is greater than in an animal exposed to the same concentrations for 5 days. ×15. Compare Fig. 8.)

FIG. 29.—Exp. 10, k6, showing a segment of revascularizing ingrowth after 36 days in air following 8 days' hyperoxia. Note the very profuse intravitreal ingrowth on the surface of the retina. ×15.

old, 5 days in 70–80 per cent. oxygen, and 18 days in air, whereas one kitten of the latter (Exp. 28, k1) was 8 days old, 10 days in 70–80 per cent. oxygen, and 18 days in air, so that the only experimental variant was the double exposure to oxygen in the case of the second animal. In both animals vaso-obliteration had been complete and the new vessel ingrowth on the 18th day was of the same size (6·5 mm. diameter), but the second animal showed a much more profuse intravitreal proliferation of vessels (Fig. 28). A similar picture of profuse proliferation was seen in Exp. 10, k6, in an animal kept in hyperoxia for 8 days (Fig. 29). It would therefore appear that vaso-proliferation is more marked when the period of vaso-obliteration in oxygen is more prolonged, but since there is some degree of variation in response in individual animals, the evidence provided by these experiments is insufficient to be conclusive.

(ii) Can vaso-proliferation occur if the animal is maintained in hyperoxia?

With reference to the question of vaso-proliferation in continuous oxygen, information is provided by Exps 23 and 24. In Exp. 23, kittens of 3 days old were kept in 70–80 per cent. oxygen for 16, 20, 21, and 26 days. During this time total vaso-obliteration was induced and maintained until the animals were killed. No vaso-proliferation was seen in the injected retinae, and there was no excessive angioblastic activity to be seen in sections. One animal (Exp. 23, k4), however, showed a limited outgrowth of vessels at the disc, but was found at *post-mortem* to have pulmonary consolidation, so that, although it was in oxygen, the vaso-proliferation was probably attributable to a fall in the blood oxygen concentration.

In Exp. 24, kittens of 4 days old were kept in 70–80 per cent. oxygen for 9, 13, and 15 days; total vaso-obliteration was thereby induced and maintained until death. No vaso-proliferation or excessive angioblastic activity occurred.

We therefore conclude that, providing pulmonary complications do not interfere with the absorption of oxygen, vaso-proliferation does not occur in continuous hyperoxia.

(*b*) Effect of Re-oxygenation.—We have so far carried out one experiment (Exp. 30) to determine the effect of returning an animal in the vaso-proliferative phase to the same conditions of hyperoxia as those which originally caused total vaso-obliteration, and the subsequent effect of a second return to air after the two periods of exposure to oxygen.

Complete vaso-obliteration was produced in 4-day-old kittens by exposing them to 70–80 per cent. oxygen for 5 days. Vaso-proliferation in the form of retinal revascularization developed after transfer to air for 18 days. One animal (k2) was then returned to the original concentration of oxygen for 5 days; a definite vaso-obliterative effect was again produced, but it was considerably less marked than the complete obliteration obtained by these concentrations in the case of normal vascular complexes. Transfer to air for 5 days after this second vaso-obliterative incident (k3) enhanced the intensity of vaso-proliferation, whereas an animal (k4) kept in oxygen for a further 5 days and not transferred to air showed a reduction in proliferating vessels. In interpreting these results it is important to remember that individual animals show variation in response, and that the experimental animals were over 21 days old by the time vaso-proliferation had developed and they were replaced in oxygen, *i.e.* they had entered the age group which is normally insensitive to oxygen.

Bearing these limitations in mind, it would nevertheless appear that, although a return to hyperoxia can to some extent control the vaso-proliferation stimulated by oxygen vaso-obliteration, the inevitable return to air is accompanied by an exacerbation of the process which is attributable to the effects of the further period in oxygen.

(6) EFFECT OF HYPOXIA

Since the experiments so far described indicate a relationship between oxygen-want and vaso-formation, one might expect that the normal developing vessels in an animal subjected to conditions of hypoxia would proliferate excessively. Three experiments carried out to investigate this possibility (Exps 3, 9, 26) show the effect of continuous hypoxia (10–15 per cent. oxygen) for exposure periods of 4 days (3, k2), 6 days (9, k3), 10 days (9, k4; 10, k3), and 13 days (26, k1). The most striking finding was intense engorgement of all the retinal vessels, which gave a much more uniform appearance than is seen in the normal pattern wherein capillary development varies greatly from one area to another. The peri-arterial capillary-free zones were narrowed.

In only one kitten (Exp. 9, k4) was there any evidence of increased vessel formation, and this was confined to a small area at the temporal periphery (Fig. 30) and remained within the nerve fibre layer. All sections, however, showed a definite increase in angioblastic activity, but in no case did this extend into the vitreous.

FIG. 30.—Exp. 9, k4, showing effect of exposure to 10–15 per cent. O_2 for 10 days. In one segment of the temporal periphery vaso-formation is more active than is normally seen, but the vessels are confined to the nerve fibre layer. ×29.

There was thus no evidence that ambient hypoxia can induce intravitreal vaso-proliferation, and since an animal allowed to survive in air for 63 days after 13 days' hypoxia (Exp. 26, K2) showed no abnormality, we conclude that such increase in angioblastic activity as occurred was probably within the upper limits of normality.

An animal (Exp. 3, k3) exposed to hypoxia for 4 days, followed by 3 days' hyperoxia, followed by 3 days' air, showed the usual picture of partially re-opened vessels after oxygen vaso-obliteration, a process which had apparently been uninfluenced by the preliminary period of hypoxia.

Discussion

In attempting to explain the numerous experimental findings summarized above, it must be admitted that the problems are most complex, and that, since our present knowledge of retinal physiology and of the nature of vaso-formative stimuli in general is so limited, a discussion of the probable mechanisms involved can be no more than speculative. Nevertheless, the difficulties may be considerably simplified by focusing attention in turn upon the characteristics of the two main phases of *vaso-obliteration* and *vaso-proliferation*; in the case of the developing retinal vasculature these are the immediate and remote effects of oxygen exposure, but they may or may not be inter-related in the mechanism of their production.

(1) Vaso-Obliterative Phase.—Before discussing this phenomenon in detail there are two points to be emphasized. First, although blood concentrations were not measured in our experimental animals, it is an established fact that exposure to increased concentrations of oxygen leads to an increase in the amount of oxygen in solution in the blood; the absence of abnormality among control animals in incubators and the relationship between the constrictive phenomenon, oxygen percentage, and duration of treatment, make it entirely reasonable to suppose that this pathological vascular change, which begins in the first few hours of hyperoxia, is directly or indirectly due to the oxygen-enriched atmosphere. Some reservations must be made, however, with regard to longer periods of exposure, for continuous administration, to a point where lung damage is induced, may actually result in a subnormal oxygen content of the blood (Binger and others, 1927).

Secondly, although the effects of oxygen have been extensively investigated since its isolation by Joseph Priestley in 1775, this particular effect of vaso-obliteration has not, as far as we are aware, been previously described, either in the retina or elsewhere, so that in interpreting the phenomenon we are at present somewhat confined by the limited information provided by our own investigations. It is probable, however, that the phenomenon is closely related to the existence of the capillary-free zone around the normal retinal arteries, for vaso-obliteration as seen in our experiments begins as a widening of this area; and F. W. Campbell (1951) has already demonstrated the reverse effect, namely a narrowing of the zone on lowering the oxygen tension. To simplify the discussion, the possible mechanisms involved will be discussed under separate headings.

(*a*) *Possible Role of Vaso-Constriction.*—The literature contains numerous reports that increased oxygen, either in compressed air or in hyper-oxygenated air at atmospheric pressure, causes vaso-constriction in the central nervous system in man and animals. The extensive pertinent literature has been reviewed by Bean (1945) and will not be recapitulated here, but it is of interest to note that this effect has been demonstrated in the cerebral vessels of cats (Tinel, 1927; Wolff and Lennox, 1930; Schmidt, 1934), and that it has been observed in the retinal vessels of human adults (Rosenthal, 1939; Cusick and others, 1940; Duguet and others, 1947), and of premature babies (Lamotte, 1951; Patz and others, 1952; Huggert, 1953).

Since hyperventilation produces a similar effect, it has been suggested (Lambertsen and others, 1953) that the effect is not due to a direct constrictor action of oxygen, but to a lowered arterial carbon dioxide tension caused by respiratory stimulation from CO_2 accumulation in the cells of the respiratory centre.* Indeed, it has been shown that, when CO_2 is administered along with increased oxygen tensions, any vaso-constrictive influence which oxygen may exert on pial or retinal vessels is nullified by the dilating action of the carbon dioxide (Wolff and Lennox, 1930; Cobb and Fremont-Smith 1931; Cusick and others, 1940).

Could this particular vaso-constrictive action of oxygen alone account for vaso-obliteration? We have shown that the obliteration begins as a gradual constriction of the vessels and it is known that vaso-constriction can seriously interfere with vascular growth (Ashton and Cook, 1952); on the other hand the form of vaso-constriction under discussion does not progress to vaso-obliteration, which is a phenomenon confined to the retinal vessels. Even if one were to assume that growing vessels by reason of their immature nerve supply were more susceptible

* This is the result of interference with the buffer function of haemoglobin which necessitates the transport of CO_2 at a higher tension.

than adult vessels to constrictor influences, it would be expected from the above reports that carbon dioxide would be able to prevent the action of oxygen, whereas it was found to have no effect in concentrations of up to 5 per cent.

It would, therefore, seem most unlikely that the peculiar effect of oxygen on growing retinal vessels can be related to the known vaso-constrictive action of oxygen. Information which might further elucidate this point could be obtained by studying the effect of oxygen on extra-retinal growing vessels, and experiments along these lines are described subsequently in this journal (Ashton and Cook, 1954).

(*b*) *Toxic Effects of Oxygen.*—Investigations carried out in recent years have provided evidence that increased concentrations of oxygen inhibit enzyme activity; the literature was fully reviewed by Bean (1945) and it has since been shown that cellular enzymes which depend on labile sulphur are particularly susceptible (Stadie and Haugaard, 1945a, b; Stadie and others, 1945a, b; Dickens, 1946; Haugaard, 1946). It is thus conceivable that oxygen vaso-obliteration might be related to an interference with the retinal enzyme systems. Here again, however, it is difficult to understand why other vessels, particular those of the cerebrum, are not similarly affected or why the effect should be confined to growing retinal vessels, On the basis of our present knowledge it is not possible to explain vaso-obliteration solely by the action of oxygen on enzymes or by unknown toxic effects which have been postulated to account for the acute convulsive type of poisoning caused by oxygen at increased pressure. Furthermore, there is some doubt whether oxygen at atmospheric pressure is an enzyme poison; Stadie and Haugaard (1945a) studied the oxygen uptake of lung slices in dogs exposed for 48 hours to one atmosphere of oxygen and found it to be about normal.

(*c*) *Consideration of Structure, Function, and Development.*—Since the known actions of oxygen considered above have failed to provide a satisfactory explanation of retinal vaso-obliteration it is necessary to consider some new or additional mechanisms. The peculiar vulnerability of the growing retinal vessels might, for instance, be related to the unique anatomical and developmental relationships of the retinal and choroidal vessels. The retinal vessels of the kitten bud off from a ciliary ring of vessels at the disc and the process of vascularization spreads as a primitive capillary net; arteries and veins later differentiate behind the growing edge; growth occurs chiefly from the veins, as Michaelson (1948a, b) first pointed out. The choroid during this period appears well developed and obviously supplies the bulk of the retina's metabolic needs in front of the vascularized area. The effect of the hyaloid system of vessels (already atrophying and usually gone by 3 to 4 weeks) and those of the ciliary body on the oxygen-content of the vitreous is probably of minor importance. Here it is well to note the inter-position of a tapetal layer through the region of Bruch's membrane in the upper half of the cat's retina, for we noted no difference in effect throughout this area.

It would seem, therefore, that the choroidal circulation maintains the retinal nutrition during the early stages of development and is later supplemented by retinal vessels growing through tissue in which the oxygen tension at that time must largely be determined by the choroidal circulation. As the choroidal supply gradually becomes inadequate to meet the increasing metabolic requirements of the developing retina, of which oxygen is the most imperative, so the new vessels bud into it; it is, therefore, clear that whatever the nature of the vaso-formative stimulus, it is probably closely related to want of oxygen.

It is reasonable to suppose that raising the oxygen tension of the choroidal blood is followed by an increased oxygenation of the retina and, therefore, of the tissue into which the retinal vessels are budding; vaso-obliteration could be explained by postulating that the vaso-formative stimulus is thus removed, further vascular growth ceases, the circulation closes down, and vascular atrophy ensues. The exceptional rapidity and extent of this effect would be in accord with the known lability of developing circulations spreading in one direction and regressing in another. In this way the two phases of vaso-obliteration and vaso-proliferation may be explained as opposite manifestations of the same mechanism—the vessels either collapsing or proliferating according to the degree of disparity between oxygen supply and demand. Consideration must, however, be given to the possibility that hyper-oxygenation of the retinal tissue produces a vaso-constricting metabolite which gradually accumulates until the retinal arterioles close in spasm to be followed by passive collapse of the venous side of the circulation. That this hypothetical factor has a specific effect on growing vessels may be related to their immature nerve supply. Herein lies the starting point for future research work which may profitably be directed towards the exclusion or demonstration and identification of such a substance.

(2) Vaso-Proliferative Phase.—Vaso-formation in the retina may be normal or abnormal and the first problem that arises is whether the stimulus in each case differs in nature or only in degree. The fundamental biological phenomenon of new vessel formation has been extensively investigated but no complete agreement has been reached on the nature of the vaso-formative stimulus; the various theories as relating to corneal vascularization have recently been reviewed by Ashton and Cook (1953), and it is clear from their study that there is much to advocate tissue hypoxia, or a metabolite produced in these circumstances, as an important factor in the vascularizing process. Such a stimulus could account for the growth of both the normal developing vessels and the abnormal proliferations, and it is most probable that the difference in response is only one of degree.

This concept of a vaso-formative factor produced in circumstances of tissue hypoxia or anoxia may readily explain our experimental findings providing it is also postulated that the factor is removed or utilized as the vessels are attracted into the tissue. Thus in normal vascularization the delicate balance between stimulus and growth ensures that there is no undue accumulation of the vaso-formative factor, so that angioblastic activity cannot become excessive; vessel growth remains confined to the inner layers of the retina in which the stimulus arises, and vascular growth ceases once the tissue is adequately supplied with oxygen. The facts that this process may be accentuated to some extent by exposing the animal to hypoxia, and retarded by hyperoxia (that is, in insufficient concentrations to cause vaso-obliteration) are consistent findings.

With regard to the vaso-proliferative phase of the kitten's retina, it may be suggested that, as a result of oxygen vaso-obliteration, the retina, being in a state of active metabolism which in oxygen had been sustained by the increased nutritional range of the choroid, suffers on transfer to air an acute oxygen-lack which is much greater in degree than normal and more severe than can be induced by such hypoxic atmospheres as are consistent with survival. Since the obliterated vessels are prevented by coagulation from re-opening adequately, the vaso-formative factor accumulates in the retina, seeps into the vitreous, and may even circulate towards

the iris through the posterior chamber. Large numbers of vessels then bud into the retina from the disc or from re-opened vessels, advance wildly into the retina, and burst into the vitreous, producing a proliferative picture far beyond the upper limits of normality. At the same time new vessels may appear on the iris. Diffusion of the vaso-formative factor outwards would not take place, as oxygen from the choroid would destroy it or oppose its action. This profuse neovascularization argues strongly in favour of a positive vaso-formative factor, for the converse theory (that vascularization occurs when an inhibitory substance is destroyed) less readily explains the cumulative effect.

This concept, considered in relation to the difference found between the marked vaso-obliterative effect of oxygen upon the normal ingrowing complexes and its relatively weak action upon the vaso-proliferative phase, wherein we assume the vaso-formative factor has amassed, implies an opposing action between the mechanism of the vaso-obliteration in hyperoxia and that of the vaso-formative factor in hypoxia. Our finding that vaso-proliferation is more marked when the duration of hyperoxia is more prolonged appears to be rather contrary to this view, suggesting an accumulation of a vaso-formative factor in hyperoxia; but this is not necessarily true, for the alternative possibility, that the longer the vessels are kept obliterated the less the degree of re-opening in air, is an equally probable explanation of the variation in vaso-proliferation.

The long-term results of the vaso-proliferative phase that, once the vessels have grown into the vitreous in response to the vaso-formative factor which had diffused into it, they gradually atrophy again, presumably because the factor is destroyed and there is no cellular activity in the vitreous to maintain the stimulus. This explains why, in complete revascularization of the retina, the intravitreal proliferations are always most in evidence peripherally, having had more time to atrophy posteriorly.

In summary, it may be said that the postulation of the elaboration of a vaso-formative factor in anoxic retinal tissue is a satisfactory working hypothesis to explain the events which follow transfer to air after oxygen exposure. It is probable that this stimulus is identical with the normal in kind but excessive in degree. This should be a suitable point of departure for future research work, and the identification of the nature of this factor might well throw considerable light upon the whole problem of vascular growth.

Role of Oxygen in the Aetiology of Retrolental Fibroplasia

Before discussing the importance of oxygen therapy in the genesis of retrolental fibroplasia, it would be well to determine to what extent our experimental results parallel the pathological picture of the disease. The further studies we have carried out since the publication of our preliminary report have completely confirmed our original findings, with one important exception. It is now recognized that the retinal detachment we reported as occurring in the vaso-proliferative phase was due to artefact produced on opening the globe; histological examination has since shown that detachment does not occur in the kitten's eye, however severe the vaso-proliferation induced by oxygen exposure. Thus while the experiments have exactly reproduced the histological picture of Stage I retrolental fibroplasia, the failure of the retina to detach has prevented the experimental reproduction of Stages II and III. It is not known why the kitten's retina fails to detach; it may be that there is some fundamental difference between the human and the experimental

disease, but the lesions of the early stage are so exactly similar that an aetiological relationship seems undeniable, and we believe that the different course is more probably referable to anatomical variations between the eye of man and that of the cat. Indeed the importance of retinal detachment as a factor in determining the course of the disease is seen also in man, for many authorities have noted a similar regression of retrolental fibroplasia in cases where no extensive detachment has occurred. As in the kitten, if detachment does not occur, the vaso-proliferation expends itself and the vessels return to an approximately normal pattern. It is not necessary to discuss here every possible facet of the relationships between the disease in man and the cat, but our studies of the pathology of the conditions have led us to the firm conclusion that, although the two disorders differ in their course, they are identical in origin. That retrolental fibroplasia is largely confined to premature babies, is commonest in the smaller weight groups, and is related to oxygen exposure, is exactly paralleled in our experimental findings.

Apart from our own work, several other experimental studies relating to oxygen and ocular abnormalities have been reported in the literature. As regards low concentrations of oxygen, lesions resembling retrolental fibroplasia have been produced in the offspring of mice subjected to an anoxic insult during pregnancy (Ingalls and others, 1952). Most workers, however, investigated the effect of high concentrations of oxygen: Coxon (1952) stated that attempts to produce retrolental fibroplasia in newborn rabbits by exposing them to oxygen concentrations of 70–80 per cent. were unsuccessful, whereas Gyllensten and Hellström (1952) succeeded in producing intra-ocular haemorrhages and hyperplastic vitreous changes in newborn mice by exposing them intermittently to 100 per cent. oxygen, but these are not the fundamental changes of retrolental fibroplasia.

Since the publication of our preliminary communication, Patz and others (1953) have reported studies in which they produced retinal vaso-proliferative changes characteristic of Stage I retrolental fibroplasia by exposing newborn mice, kittens, and puppies to 70–80 per cent. oxygen for varying periods. Since the eyes of these animals were examined only histologically, the vaso-obliterative effect of oxygen was not observed, but in a more recent investigation Patz (1954) employed the Indian ink injection technique on rats and dogs. He noted that animals exposed to oxygen concentrations of 40–50 per cent. showed a marked retinal vaso-constriction, whereas concentrations of 70–80 per cent. induced complete obliteration of the vessels after 3–5 days' exposure. These findings thus completely confirm our original report. In addition, however, Patz described the appearance of a pathological degree of angioblastic activity developing while the animals were still in continuous oxygen. As stated elsewhere, however, we would attribute this rather to a fall in blood oxygen concentration through pulmonary changes, for we have found that in animals remaining healthy in hyperoxia the contraction or obliteration of the retinal vessels is maintained; in these circumstances vaso-proliferation is not to be expected, nor, in our experience, does it occur.

The clinical evidence incriminating oxygen is considerably more extensive than the experimental work, and although the reports appear conflicting they are curiously more conclusive for that reason.

The literature has been well reviewed by Zacharias (1952) and need not be repeated here, but it is of importance to stress that none of the oxygen theories of pathogenesis can now be completely accepted, because when they were advanced

the actual effect of oxygen on growing retinal vessels was completely unknown. Now that the vaso-obliterative action of oxygen has been demonstrated in the retinal vessels of the kitten, and we have reason to suppose that the human eye may be similarly affected, many of the conflicting clinical opinions become reconcilable. Thus, the fact that there are two essential components of the disease (the one occurring in high oxygen concentrations, and the other, dependent on the extent of the first, developing in air) resolves the paradox of anoxic conditions resulting from high oxygen levels, without postulating a histotoxic anoxia, and brings together the apparently opposing views that the disease may be due either to hyperoxia or to hypoxia. Nor is it surprising that returning babies to oxygen has been found to be beneficial, for, as we have shown, the proliferating vessels are once again obliterated, although to a considerably less extent than originally.

The reported association of retrolental fibroplasia with cerebral palsy, epilepsy, and mental deficiency is not, however, explained by the vaso-obliterative effect of oxygen, which is apparently confined to the retinal vessels; this may possibly be due to a toxic effect, but one would have thought that the ordinary vaso-constrictive action of oxygen might well interfere with the high oxygen supply required for the normal development of the cells of the central nervous system.

Since it is now apparent that the vaso-proliferative phase of retrolental fibroplasia is due to retinal anoxia or hypoxia, some reference should be made to the possibility that low blood concentrations of oxygen, whether in the foetus or premature infant, may not directly produce the disease. Provided an oxygen-lack as severe as that arising with oxygen vaso-obliteration may occur in such circumstances, there would appear to be no reason why this should not occur, for even in the adult eye vaso-proliferation is a common accompaniment of those conditions in which the retinal vessels are occluded or destroyed, such as diabetic retinopathy, central venous occlusion, or Eales's disease. Our experiments, however, suggest that low oxygen atmospheres consistent with survival are not able to induce a sufficient degree of retinal hypoxia in the normal developing eye to give rise to vaso-proliferation into the vitreous. This finding is in accord with the fact that there is no association between retrolental fibroplasia and pulmonary or cardiac disease. On the other hand it is possible that much greater degrees of anoxia may occasionally occur *in utero* and derange vascularization. While this possibility remains to be further investigated, it is to be remembered that neither the necessity for resuscitation (Ryan, 1952), nor the presence of cyanosis (Krause, 1946; Ryan, 1952; Unsworth, 1949), nor asphyxia (Krause, 1946) appear to have any correlation with the subsequent development of retrolental fibroplasia.

Thus the clinical, pathological, and experimental evidence justifies the recognition of the primary importance of hyperoxia in the genesis of retrolental fibroplasia, but this conclusion should not obscure the need to seek for other possible contributory factors, the existence of which is suggested by the fact that the disease is not found in all premature infants exposed to high concentrations of oxygen. On the other hand, this variation may be due simply to individual idiosyncrasy, for we have shown that in kittens of the older age group, vaso-obliteration, which is then confined to the periphery, occurs in only a proportion of the test animals.

Considering our experimental results in relation to *therapy*, it appears that the greatest hope lies in prophylaxis ; once the dangers of oxygen are appreciated by all responsible for the care of premature infants, so that it is administered only when

essential and then only in the smallest quantity and for the shortest time consistent with the infant's survival, the disease should disappear or its incidence should be reduced to relatively insignificant proportions. The recent report of Locke (1954) gives good grounds that this hope will be realized; he noted a striking fall in the incidence of the disease when the routine administration of oxygen is reduced to the minimum—it occurred in only two out of 124 babies (both mild cases) when, on the basis of previous experience, 32 cases were to be expected.

The exact amount of oxygen that may be given to a premature baby with impunity cannot, of course, be gauged by experiments on normal kittens, but the fact that concentrations below 35 per cent. have proved innocuous to these animals should be a guide. We have no experimental data on the value of graduating the withdrawal from oxygen, which has been advocated as a valuable method in preventing the disease (Szewczyk, 1951), but it is difficult to see how a gradual or rapid withdrawal could influence the course of events determined by vaso-obliteration and intravascular coagulation once they have occurred. Since we have found that Tromexan allows the retinal vessel to re-open, it appears much more likely that anticoagulants offer the best preventive therapy where the risk of exposure to high oxygen concentrations must be taken.

Once the vaso-proliferative phase of the disease has begun the only apparent rational therapy is to attempt to reduce the angioblastic activity. Szewcyk (1951) recommended returning the infant to oxygen, and reported a dramatic improvement in nine cases with this treatment. While we found that re-oxygenation partially obliterated the proliferating vessels or retarded their growth, the inevitable return to air was accompanied, as was to be expected on theoretical grounds, by an exacerbation of the process, thus suggesting that such treatment of human cases may not be without its dangers.

The use of ACTH and cortisone, although originally employed on a different basis, should theoretically be of value in controlling the early stages of retrolental fibroplasia, for it is established that both hormones exert a considerable inhibitory influence on the formation of new vessels (Duke-Elder and Ashton, 1951; Ashton and Cook, 1952). Unfortunately the results have been generally discouraging, and the side-effects of these hormones appear to be particularly dangerous to the premature baby. Once a marked degree of retinal detachment has occurred the disease is irreversible and no treatment is known which can modify the subsequent developments.

In view of the tragic results of retrolental fibroplasia and the inadequacy of available treatment, the prevention of the disease is urgent and imperative; on the basis of present knowledge it appears that the problem is fortunately a simple one, being mainly a matter of ensuring that those units concerned are fully aware that oxygen, administered to premature babies, is to be regarded as a dangerous drug.

Summary

(1) High concentrations of oxygen at atmospheric pressure are able to induce in the growing retinal vessel of the kitten a type of vaso-constriction which progresses to complete obliteration of the vessels. This newly-described phenomenon has not been observed in extra-ocular vessels, and is apparently confined to the developing retina. It occurs also in the ratling and young rabbit, but to a much less extent.

(2) The severity of vaso-obliteration is inversely proportional to the maturity of the vessels, and obliteration cannot be produced once the retinal vasculature is fully mature; it is directly related to the duration of exposure to oxygen, and it takes about 36 hours, as judged by injection preparations, to obtain total obliteration in the most sensitive age groups; it is directly related to the concentration of oxygen, and concentrations below 35 per cent. have little or no effect in the most sensitive age groups.

(3) On transfer to air the obliterated vessels only partially re-open, producing haemorrhages and a grossly abnormal vascular pattern. The vascular closure is rendered permanent by thrombosis or adherence of the opposing endothelial cells through plasma coagulation. Histologically the obliterated vessels are represented by clusters of endothelial cells, which, unless the vessels re-open adequately, eventually disappear.

(4) This partially re-opened network is inadequate to nourish the retina, and profuse vaso-proliferation develops. When obliteration has been total the retina is revascularized from the disc and those vessels that re-opened disappear; the new ingrowth, which is not arranged in the normal three complexes and consists of a dense circular mass of vessels, extends into the retina in a profuse and disordered manner, and glomerular tufts and vascular loops grow into the vitreous. Eventually the whole retina is revascularized, arteries and veins differentiate, and the intravitreal vessels gradually disappear in a postero-anterior direction. Retinal detachment does not occur, and, apart from acute-angled folds in the retina, the normal histology is restored and vision is retained. The fundus picture, however, is grossly abnormal, there being an irregular vascular pattern and many more main vessels around the disc than normal. Where obliteration has been partial, vaso-proliferation does not originate from the disc region only, but arises from the whole field of unobliterated or re-opened vessels; in other respects the picture is similar. Where obliteration has affected only the periphery of the retinal vasculature, then vaso-proliferation and intravitreal growth is similarly confined to this region, the posterior fundus being normal—a picture which exactly parallels that seen in most cases of Stage I retrolental fibroplasia. Vaso-proliferation did not occur in rabbits exposed to hyperoxia.

(5) Several factors which might influence the above course of events were studied:

(*a*) In prolonged hyperoxia, it was found that obliterated vessels remain closed as long as the animal is kept in oxygen, providing no pulmonary complications ensue, and that if high concentrations of oxygen are maintained no vaso-proliferation occurs.

(*b*) No difference in the degree of vaso-proliferation was demonstrated on transferring animals to hypoxia rather than to air.

(*c*) Carbon dioxide in concentrations up to 5 per cent. was found to have no effect on either the vaso-obliterative or vaso-proliferative phases.

(*d*) Priscol, in a single experiment, did not appear to modify the vaso-obliterative effect of oxygen.

(*e*) It was found that Tromexan profoundly modified the degree of vaso-obliteration seen after oxygen exposure; this is probably not due to an antagonism to oxygen but to the prevention of intravascular coagulation so that affected vessels are able to re-open in air.

(*f*) In studying the effect of re-oxygenation, it was found that, although a return to hyperoxia can to some extent control the vaso-proliferation stimulated by oxygen vaso-obliteration, the inevitable return to air is accompanied by an exacerbation of the process, and that this is attributable to the further period in oxygen.

(6) Normal animals subjected to hypoxia showed a definite increase in retinal angioblastic activity, but intravitreal proliferations did not appear either in hypoxia or on transfer to air.

(7) The probable mechanisms involved in the vaso-obliterative and vaso-proliferative phases are discussed, and reasons are given for supposing that vaso-obliteration cannot be explained solely in terms of the known vaso-constrictive or toxic action of oxygen. The limitation of this phenomenon to growing vessels may be attributable to the structural and functional relationships of the retinal and choroidal circulations which permit hyperoxygenation of the retina; thus the normal vaso-formative stimulus may be destroyed and the growing vessels may then collapse and atrophy; alternatively, a specific vaso-constricting metabolite with the power to obliterate immature vessels may be produced.

The features of the vaso-proliferative phase point to the production of a vaso-formative factor, elaborated in circumstances of tissue hypoxia or anoxia, which is removed or utilized as the vessels grow into the tissue. It is probable that this stimulus is identical with the normal in kind but excessive in degree. This concept explains all the events which develop in the eye on transfer to air after oxygen exposure, and may be applied to the problem of vaso-proliferation in such conditions as diabetic retinopathy, central venous occlusion, and Eales's disease.

It is pointed out that the above suggestions are no more than a working hypothesis, but future research may profitably be directed towards the identification or exclusion of such vaso-obliterative or vaso-formative factors.

(8) Relating these experimental results to retrolental fibroplasia, it is concluded that the findings exactly parallel the early stages of the human disease. The difference in the subsequent course is dependent upon the development of retinal detachment which did not occur in the kitten experiments. Despite this difference the two diseases appear identical in origin and this conclusion is supported by other experimental work and by considerable clinical evidence.

(9) Considering the results in relation to treatment it is apparent that prophylaxis is by far the most hopeful line of attack and an urgent plea is made for the control of oxygen therapy in the treatment of the premature baby.

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APPENDIX

EXPERIMENTAL DATA

Further details of our experimental conditions and results are shown in Tables II and III.

The following abbreviations are employed:

Vaso-obliteration, degree indicated by plus signs, ++++ being total.
Vaso-proliferation, degree indicated by plus signs, ++++ being most profuse.
} As seen in injected retinae

RD Revascularization from disc with intravitreal vessels.
P Peripheral intravitreal vessels
G Generalized intravitreal vessels

TABLE II

EFFECT OF HIGH CONCENTRATIONS OF OXYGEN

Oxygen Concentration (per cent.)	Experiment No.	Age (days)	Exposure (days)	Air (days)	Age at Death (days)	Vaso-obliteration	Vaso-proliferation
	32, k1	2	½		2½	++	0
	k2	2	1		3	+++	0
	k3	2	1½		3½	++++	0
	2, k1	2	Control		3	0	0
	k2	2	3		5	+++	0
	k3	2	3 (died)		5	+++	0
	1, k1	3	4		7	++++	0
	k2	3	4		7	++++	0
	k3	3	6		9	++++	0
	23, k1	3	16		19	++++	0
	k2	3	20		23	++++	0
	k3	3	21		24	++++	0
	k4	3	26		29	++++	+ (pneumonia)
70–80 without survival in air	24, k1	4	15		19	Sections only	Sections only
	k2	4	13		17	++++	0
	k3	4	9		13	++++	0
	11, k1	17	4		21	+	0
	k2	17	Control		21	0	0
	25, k1	18	10		28	++	0
	k2	18	10		28	++	0
	13, k1	29	7		36	±	0
	k2	29	12		41	0	0
	k3	29	Control		32	0	0
	14, k1	50	16		66	0	0
	k2	50	7		57	0	0
	k3	50	Control		57	0	0
	8, k1	58	5		63	0	0
	k2	58	Control		63	0	0

TABLE II—*continued*

Oxygen Concentration (per cent.)	Experiment No.	Age (days)	Exposure (days)	Air (days)	Age at Death (days)	Vaso-obliteration	Vaso-proliferation
70–80 with and without survival in air	7, k1	2	Control		16	0	0
	k2	2	5	9	16		++ RD
	k3	2	5	30	37		+++ RD
	k4	8	Control		31	0	0
	k5	8	5		13	++++	0
	k6	8	5	18	31		+++ RD
	12, k1	3	4	65	72 (died)	Sections only	Sections only
	k2	3	4	65	72		+++ RD
	k3	3	4	65	72 (died)	Sections only	Sections only
	k4	4	4	Still alive	Still alive		+++ RD ++ P
	k5	4	4	65	73		+++ RD +++ P
	k6	4	4	151	159		+++ RD ++ P
	28, k1	8	10	18	36		++++ RD
	27, k1	12	4		16	+++	0
	k2	12	4		16	+++	0
	k3	12	4	21	37		++++ G
	36, k1	18	10		28	+ (peripheral)	0
	k2	18	10	18	46		+++ P
	k3	18	10	31	59		0
	k4	18	13		31 (died)	+	0
	k5	18	20		38	0	0
	19, k1	22	5	23	50		0

TABLE III

EFFECT OF OTHER CONCENTRATIONS OF OXYGEN

Oxygen Concentration (per cent.)	Experiment No.	Age (days)	Exposure (days)	Air (days)	Age at Death (days)	Vaso-obliteration	Vaso-proliferation
35–45 without survival in air	16, k1	4	4	1 (died)	9	+++	0
	k2	4	3		7	+++	0
	k3	4	4	1 (died)	9	+++	0
	k4	4	3 (died)		7	Sections only	Sections only
	k5	4	4	1 (died)	9	+++	0
25–35 with and without survival in air	29, k1	3	5		8	0	0
	k2	3	7		10	0	0
	k3	3	7		10	Sections only	Sections only
	35, k1	4	21		25	0	0
	k2	4	20		24	Sections only	Sections only
	k3	4	21		25	0	0
	15, k1	1	7		8	±	0
	k2	1	14	33	48		0
	k3	1	14	75	90		0
	k4	1	14		15	0	0
10–15 with and without survival in air	9, k1	4	Control		4	0	0
	k2	4	Control		14	0	0
	k3	5	6		11	0	0
	k4	4	10		14	0	+
	k5	4	1 (died)		5		
	26, k1	4	13		17	0	0
	k2	4	13	63	80		0
	k3	4	10		14	0	0

TABLE IV

EFFECT OF EXPOSURE TO OXYGEN COMBINED WITH OTHER TREATMENT

Test	Experiment No.	Age (days)	Oxygen (per cent.)	Exposure (days)	Air (days)	Age at Death (days)	Vaso-obliteration	Vaso-proliferation
Various degrees of oxygen exposure, then CO_2, Priscol, or Tromexan	17, k1	9	60–70	4	41	54		+++ RD
	k2	9	then	4		13	++++	0
	k3	9	5 per cent. CO_2	4	42	55		+++ RD
	31, k1	12	70–80	5 with Priscol		17	+++	0
	k2	12		5		17	+++	0
	18, k1	14	70–80	5	2	21	++	0
	k2	14		5 (Tromexan)	2	21	+	0
	20, k1	8	70–80	3 (Tromexan)		11	++	0
	k2	8		3 (Tromexan)		11	Sections only	Sections only
	k3	8		3		11	++++	0
	21, k1	8	45–55	4		12	+++	0
	k2	8		4 (Tromexan)	3	15	+	0
	k3	8		2 (died)		10	Sections only	Sections only
	k4	8		1 (died)		9	Sections only	Sections only
	k5	8		4 (Tromexan)		12	+	0
	22, k1	13		Control		13 (died)	Normal	0
	k2	13	70–80	4 (Tromexan)		17	+++	0
	k3	13		4 (Tromexan)	2	19	++++	0
35–45 per cent. oxygen, then 70–80 per cent. oxygen, with survival in air	10, k1	3	35–45	4		7	+++	0
	k2	3		4		7	++	0
	k3	3		Control		7	0	0
	k4	3		Control		7	0	0
	k5	3	35–45 for 4 days; then 70–80 per cent. for 4 days, then 63 days' air			74		+++ RD ++ P
	k6	3	35–45 for 4 days; then 70–80 per cent. for 4 days; then 36 days' air			47		++++ RD
	k7	3		Control		76	0	0
	k8	3		Control		48	0	0
10–15 per cent. oxygen, then 70–80 per cent. oxygen, with survival in air	3, k1	1		Control		1	0	0
	k2	1	10–15	4		5	0	0
	k3	1	10–15 for 4 days; then 70–80 per cent. for 3 days; then 4 days' air			12	++	0
70–80 per cent. oxygen, then 10–15 per cent. oxygen, with survival in air	4, k1	1		Control		1	0	0
	k2	1		Control		5	0	0
	k3	1		Control		8	0	0
	k4	1	70–80	4		5	++++	0
	k5	1	70–80 for 4 days; then 10–15 for 3 days			8	++++	? (disc region obscured)
	k6	1	70–80	4	3	8	++++	+ RD
70–80 per cent. oxygen, then survival in air, with reoxygenation (70–80 per cent.)	30, k1	1	70–80	5	18	24		+++ RD
	k2	1	70–80 for 5 days; then 18 days air; then 70–80 per cent for 5 days			29	+	+++ RD
	k3	1	70–80 for 5 days; then 18 days air; then 70–80 per cent. for 5 days; then 5 days' air			34		++++ RD ++ P
	k4	1	70–80 for 5 days; then 18 days air; then 70–80 per cent. for 10 days			34	+	++++ RD + P

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DIRECT OBSERVATION OF THE EFFECT OF OXYGEN ON DEVELOPING VESSELS

PRELIMINARY REPORT

BY

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In previous papers it has been shown that high concentrations of oxygen are able to exert on growing retinal vessels a peculiar type of constrictor action which progresses gradually to complete obliteration of the ingrowing complexes (Ashton and others, 1953, 1954). The possible factors involved in this mechanism have already been fully discussed; it is the purpose of this paper to report preliminary experiments designed to elucidate the problem further.

In order to study the process of vaso-obliteration and to correlate the findings in injected retinae with events in life, we have utilized a new limbal window technique, devised by one of us (C.C.), which permits direct observation of the living retinal vessels. To determine whether oxygen has a similar action on growing vessels in the adult animal we have investigated the effect of hyperoxia on vascularization of the rabbit cornea and rabbit ear chamber; indirect evidence for its action on vessels in the embryo has been sought in a few experiments on hens' eggs. The methods employed together with the results obtained will be reported under the appropriate headings.

(1) Oxygen and Corneal Vascularization

The rabbit was used because a method of inducing corneal vascularization by the intracameral injection of alloxan was available (Langham, 1953), and it had been shown that the vaso-obliterative phenomenon occurs in the developing retinal vessels of this animal (Ashton and others, 1954). All the animals in two series of experiments were injected with alloxan according to the above technique.

In the first series of six animals, three served as controls, whilst the remaining three, on the 6th day after the alloxan injection, by which time a well marked peripheral zone of corneal vascularization was present, were placed in an oxygen chamber maintained continuously at 70–80 per cent. oxygen.

In the second series of six animals, three served as controls, whilst the remaining three animals were placed in the oxygen chamber immediately after the alloxan injection.

The extent and density of the new vessel formation was examined daily and compared with that present in the control animals over a period of 6 to 7 days. There was no difference in either experiment between the control and test animals; thus oxygen in concentrations of 70–80 per cent. apparently *had no vaso-obliterative effect on growing vessels in the rabbit cornea*, and did not noticeably affect the rate of vascular growth.

(2) Oxygen and New Vessel Formation in the Rabbit Ear Chamber

Perspex chambers were inserted into one ear of five rabbits by a technique similar

to that of Sandison (1928). A well-marked capillary ingrowth was present at the periphery of the chamber 14 days after they were inserted. A series of photo-micrographs was taken of selected areas of the vascular ingrowth, and the rabbits were then placed in a gas chamber maintained continuously at 70–80 per cent. oxygen. The ear chambers were re-examined daily and serial photomicrographs were taken of the chosen areas over a period of 4 to 5 days. All the animals died suddenly at the end of this time. During microscopical examination pure oxygen was administered to the animals through a closely fitting mask; in one animal the microscopical appearance of the ear vessels was observed continuously over a period of 10 hrs from the commencement of oxygen administration.

There was no evidence of vaso-constriction or vaso-obliteration, or of any significant alteration of the rate of vascular growth.

(3) Direct Observation of the Developing Retinal Vessels of the Kitten

To observe the effect of oxygen on the developing retinal vessels in the living animal and to determine the time relationships of the vaso-obliterative phenomenon, a new limbal window technique was employed. The method is as follows:

The animal is anaesthetized with intraperitoneal Nembutal. Both the upper and lower lids and the nictitating membrane are then removed and the conjunctiva is incised around the limbus and reflected to expose a 3 mm. ring of sclera. Four equidistant sutures are inserted into the sclera 2 mm. behind the limbus and the ends are left free. A metal ring 9–10 mm. in diameter, which encircles the limbus, is then attached to the globe by means of the sutures which pass through four perforations in the rim. The entire cornea is then removed either by cautery or scissors and the iris is completely abscised. It has been found that haemorrhage can be almost completely prevented by application of the cautery to the base of the iris prior to its removal.

The anterior capsule of the lens is then excised with scissors and the soft lens matter gently removed by curette and irrigation. The posterior capsule and posterior portion of the tunica vasculosa lentis should be left intact, for if they are broken a retinal detachment rapidly occurs. A circular glass cover-slip of the appropriate size is then inserted within the ring so that it rests upon a narrow ledge ground into its rim; it is held firmly in place by means of a circular wire clip which is similarly inserted into the ring to rest upon the cover slip (Figs 1 and 2).

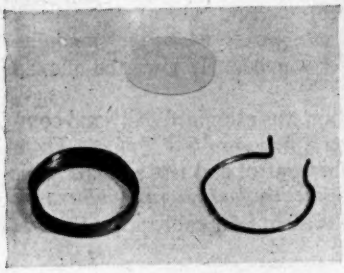

FIG. 1.—Components of limbal window: recessed metal ring, glass disk, and spring clip. ×1·5

FIG. 2.—Limbal window *in situ*. Even at this magnification the retinal vessels may be seen in the fundus. ×5

The kitten is now placed in a rectangular Perspex box in which an inlet and outlet tube has been inserted for the purpose of administering oxygen. At one end of the top of the box is drilled a small hole, so situated as to correspond with the position of the ring attached to the eye when the animal's head is held in close apposition to the top of the Perspex box by means of a linen sling. Through this aperture the retinal vessels can now be observed microscopically *via* the limbal window whilst the oxygen is being administered.

The illuminating system consists of a mercury vapour lamp and a condensing system which directs a convergent beam of light onto a small, plane reflecting mirror placed within the focal length of the condensing system. A divergent beam is thus reflected into the eye, the area of retina illuminated being controlled both by the position of the reflecting mirror and by a small iris diaphragm, which, together with a plate of heat absorbing glass (Chance ON 20), is situated between the source of light and the condensing lens.

The technique has the great merit of permitting examination and photographic record of the living retinal circulation, the smallest vessels of which may be seen in their finest detail. It has the disadvantage of seriously interfering with the structure of the eye so that retinal detachment may occur; the possible effects this may have on normal function must be borne in mind in interpreting the experimental findings.

Results.—Seventeen experiments have been carried out, involving 22 kittens and one cat. All showed the same general changes. Four representative experiments are quoted below.

EXPERIMENT 1 (52).—An adult cat maintained in 80 per cent. oxygen showed no changes in the retinal vessels as observed through a limbal window over a period of 1½ hrs (Fig. 3).

FIG. 3.—Experiment 1, fundus of adult cat photographed through limbal window Oxygen had no observable effect on the retinal vessels. ×17

EXPERIMENT 2 (51).—In a kitten (11 days old) after 3-5 minutes exposure to 80 per cent. oxygen the capillaries completely closed and the main vessels were severely constricted (Figs 4 and 5). This picture did not progress to total vaso-obliteration and the vessels began to re-open after about 10 minutes, although the oxygen concentrations were maintained.

FIG. 4.—Experiment 2, normal kitten retina showing arteries, veins, and capillaries. × 12. (Compare Fig. 5).

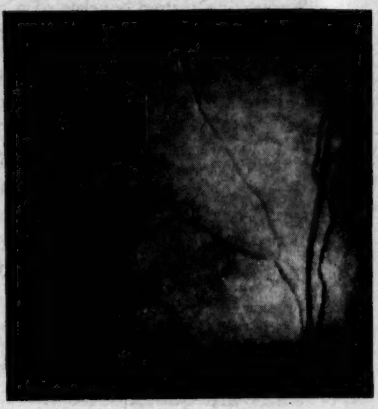

FIG. 5.—Same retina as that shown in Fig. 4 photographed 5 minutes after animal was exposed to 80 per cent. oxygen and showing the *immediate* effect of oxygen. The capillary bed has completely closed and the main vessels, particularly the arteries, are severely constricted. After 10 minutes' exposure the vessels re-opened. ×12

On return to air the main vessels dilated and the capillary bed re-opened in 5–10 mintues.

On return to oxygen the same effect as that produced initially occurred more promptly but in the same manner; again, however, the main vessels were not obliterated and continued to carry an active circulation for as long as 4 hrs.

EXPERIMENT 3 (53).—A kitten (12 days old) was pre-treated in a gas chamber (70–80 per cent. oxygen) for 4 hrs before a limbal window was inserted, oxygen being maintained during operation. When the retina was examined microscopically it was found that the whole vascular bed was open although the arteries were constricted. After a total exposure to oxygen of 5½ hrs the capillary bed began to close, and it had disappeared in 6¾ hrs. In 7½ hrs the arterial side of the circulation had disappeared; the veins were still visible but constricted.

After 8 hrs' exposure the entire retinal circulation had completely closed.

Amyl nitrite administered with the oxygen at this stage had no effect on the closed circulation.

On return to air the circulation began to re-open within 5 minutes in the order of veins, arteries, and capillaries, reaching a maximum within about 30 minutes; this was not as complete as when the animal was first examined, which suggests that the vaso-obliteration was now not entirely reversible.

On return to oxygen a complete closure developed in 7 minutes.

On return to air the vessels re-opened as previously in 5 minutes.

A second return to oxygen again led to complete closure within 5 minutes. (At this stage it was noted that a further injection of Nembutal did not affect the picture).

On return to air the vessels opened as before.

A mixture of oxygen and 5 per cent. carbon dioxide was now given, and this led to an identical closure within 6 minutes.

Raising the temperature of the box from 27° to 37° C. had no effect.

After reopening in air, vaso-obliteration was again induced by returning the animal to oxygen, and Priscol (15 mg.) was given intra-peritoneally; the vessels then slowly re-opened to the maximum degree obtained on the first transfer to air.

Transfer to air showed no further opening up of the capillary bed (Fig. 6*a*, *b*, *c*, *d*).

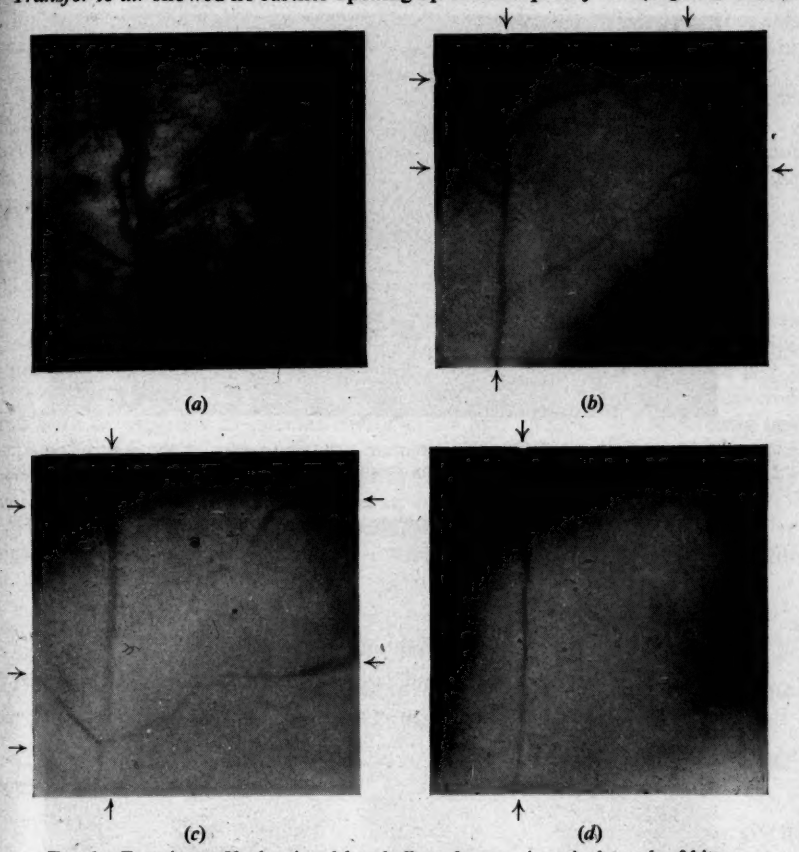

FIG. 6.—Experiment 53, showing *delayed* effect of oxygen in retinal vessels of kitten continuously exposed to 80 per cent. oxygen. ×17

(*a*) After 4½ hrs' exposure all vessels still patent.

(*b*) After 6¼ hrs main vessels constricted and capillary bed not visible.

(*c*) At a later stage main arteries began to close. Horizontal main arterial branches still faintly seen.

(*d*) After 8 hrs' exposure only one main vein could still be discerned. Soon this too disappeared, leaving a completely avascular white retina.

EXPERIMENT 4 (45).—A kitten (12 days old) pre-treated with oxygen in a gas chamber (70–80 per cent. oxygen) for 5½ hrs, was found on examination through the limbal window to have total vaso-obliteration. Retinal detachment, however, developed in one area, and the vessels in the detached portion re-opened while in the attached retina they remained closed.

Return to air re-opened all the capillaries.

Return to oxygen closed only the vessels in the attached portion of the retina (Figs 7 and 8). Eventually the whole retina became detached and all the vessels re-opened and remained fully patent for 7 hrs in 90 per cent. oxygen.

FIG. 7.—Experiment 4, kitten retina in air. The white reflex on the right of the picture is at the peak of a developing localized detachment. ×25

FIG. 8.—Same retina as that shown in Fig. 7 photographed after exposure to 80 per cent. oxygen. Note that the capillary bed has disappeared only in the attached retina on the left, the vessels being unaffected in the detached area on the right. ×25

Summary of Findings.—It must be emphasized that any conclusions presented at this early stage of our experiments must of necessity be provisional, but on the basis of present observations the following impressions have been gained.

Oxygen has no observable effect on the retinal vessels of the adult cat. On developing retinal vessels it appears to produce immediate and delayed effects. The *immediate effect* is one of constriction of the large vessels and obliteration of the capillaries, which develops 5 minutes after exposure to hyperoxia. After about 10 minutes the vessels dilate again, and the capillaries re-open and remain approximately in this state for about 5½ hrs, when the *delayed effect* comes into operation. The capillary bed again begins to close, the main vessels constrict, and after about 8 hrs' hyperoxia the whole retinal circulation is completely obliterated. Once this has been achieved the circulation may be either opened or closed, in each case in about 5 minutes, by alternating air and oxygen.

Total vaso-obliteration is apparently not affected by amyl nitrite, 5 per cent. carbon dioxide, or small rises in temperature.

Vaso-obliteration cannot be induced in the detached retina, and if detachment develops in an area of the retina in which vaso-obliteration has been induced, the vessels re-open, that is, providing that the obliteration is still reversible.

(4) Oxygen and the Developing Chick Embryo

Our experiments studying the effect of oxygen on the developing chick are as yet too inconclusive to give more than a preliminary note. So far three chicks have hatched out alive after being subjected to 80–90 per cent. oxygen during the last 7 days of incubation. They were weaker than normal but gradually recovered and were as vigorous as normal chicks in 24 hrs. Vision was apparently unimpaired and there were no defects which would point to any abnormality in the nervous, respiratory, or cardio-vascular systems. The chicks developed normally and remained healthy during a period of 6 weeks' observation.

The only conclusion permissible at this stage is that chick embryos may survive high concentrations of oxygen, hatch out alive, and develop normally in air for at least 6 weeks.

Summary

(1) Preliminary reports are given of experiments designed to elucidate the problem of vaso-obliteration by oxygen.

(2) It was found that hyperoxia has no effect on growing vessels in the rabbit cornea or in the rabbit ear chamber.

(3) A new limbal window technique is described and an account is given of direct observation of the effect of oxygen on the retinal vessels by this method. It was found that:

(*a*) Oxygen has no observable effect on the retinal vessels of the adult cat.

(*b*) In the kitten *immediate* and *delayed* reactions are seen. The former consists of vaso-constriction and capillary obliteration developing after 5 minutes

exposure to oxygen. This does not progress to total obliteration and the vessels re-open after about 10 minutes and remain in this state for about 5½ hrs when the delayed effect comes into operation. The vessels then again constrict and total vaso-obliteration gradually develops, becoming complete in about 8 hrs. Once this is achieved the circulation may be opened or closed in about 5 minutes by alternating air and oxygen.

(*c*) Vaso-obliteration cannot be induced in the detached retina, and obliterated vessels re-open when detachment develops.

(4) Chick embryos may survive 80–90 per cent. oxygen during the last 7 days of incubation, hatch out alive, and develop normally in air.

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BOOK REVIEWS

Retinal Circulation in Man and Animals. By I. C. MICHAELSON. 1954. Pp. 146, 95 figs. Thomas, Springfield, Ill. (50*s*.).

Michaelson's work on the retinal circulation both comparative and pathological is well known, and it is with pleasure that we have received this short monograph on the development and anatomy of this important part of the circulation in vertebrates. The study is essentially based on the appearances delineated by injections of Indian ink into the heart, the carotid, or the ophthalmic artery (depending on the size of the animal) soon after death, and is supplemented in many cases by ophthalmoscopic examination during life. Each class of vertebrate is studied in this way, particular attention being paid to such representative species as the eel, roach, frog, pigeon, horse, cat, rat, and man. The pattern of vascularization varies considerably among different vertebrates: in some (guinea-pig), the retina is almost avascular and its tissues are nourished almost entirely by diffusion from the choroid; in others (eel), there is no vascular choroid and the retinal vessels are maximally developed; in others (birds), a specially vascular structure, the pecten, contributes largely to the nutritional supply; and in all, many points of interest arise. Not the least important part of the work is the author's observations on the growth of new vessels and the comparison which may be drawn between the normal picture in various animals and some of the pathological changes which may occur in man. The book is original and in every sense interesting; incidentally, it is beautifully produced.

Abstracts on Military and Aviation Ophthalmology and Visual Sciences, Volumes I and II. By CONRAD BERENS and L. BENJAMIN SHEPPARD. 1953. Pp. 539 and 245. Biological Sciences Foundation, Washington, D.C.

These two volumes form a very useful summary of the literature on military and aviational ophthalmology, the first abstracting relevant papers and books from the sixteenth century until 1940 and the second summarizing the enormous amount of work published in the years 1941 to the end of 1945. The immense impetus given to the study of visual capacity owing to the increase in the speed and performance of aircraft, the growing complexity of the machines of war, and the increased strains put on the sensory apparatus, now stretched to the limits of its capacity and beyond in the attainment of supersonic speeds, account for the fact that the two volumes are about equal in size, though one covers four centuries and the other only five years.

Each volume is divided into 93 sections comprising every aspect of ophthalmology which can be even remotely associated with military and aviational activities. The subjects include such diverse topics as physiological studies of the light sense and the colour sense, methods of testing vision and the various standards which have been proposed, and the influence of such factors as glare, fatigue, drugs, refractive errors, and muscular imbalance upon visual performance. Much space is given to the literature concerned with visual environmental stresses such as high altitude, decompression, acceleration, and deceleration. Much of the available information on such topics is scattered and difficult of access, though much of it is of unusual importance and interest. The authors have done a great service in gathering it all together in a readily accessible form.

The Brain and the Eye. (Gehirn und Auge). By R. BING and R. BRÜCKNER. 1954. Pp. 248, 147 figs. Schwabe, Basel. (*Swiss Frs* 28; 50*s*.).

This is a short and concise handbook on ophthalmic neurology. The book opens with a short description of the nervous organization of the visual system—the peripheral sensory apparatus of the eye, the reflexes associated with its various activities (pupillary, labyrinthine, cortical, etc.), its motor activities, the visual pathways and centres, and the associated autonomic system. The main part of the book, which discusses clinical pathological conditions, is divided into sections: motor anomalies, sensory and trophic disturbances, diseases affecting the visual pathways from the chiasma to the cortex, and diseases of the brain and nervous system which affect the visual system secondarily or incidentally; a final section describes diagnostic methods of ophthalmological interest for elucidating central nervous diseases—a comprehensive section including the clinical examination of the retina, the pupillary reflexes and accommodation, ocular movements and nystagmus, perimetry, exophthalmos, lacrimal secretion, and so on. The book is written simply and clearly as for the senior student or practitioner, the illustrations are unusually clear and adequately explain the text, and the comfortable impression is conveyed that ophthalmic neurology is after all not so formidable a subject as may sometimes have been thought.

NOTES

OPHTHALMOLOGICAL SOCIETY OF THE UNITED KINGDOM

74th Annual Congress, 1954

The Society's 74th Annual Congress was held at Newcastle-upon-Tyne, under the presidency of Mr. Alexander MacRae, on April 22, 23, and 24. One hundred members were present. After a short introductory speech by the President, the Nettleship Medal was presented to Dr. Norman Ashton, Director of Pathology at the Institute of Ophthalmology, London, in recognition of his valuable contributions to ophthalmology, his study of the anatomy of Schlemm's canal, of the aqueous veins, and of the choroidal circulation by the use of Neoprene casts being particularly mentioned.

The main discussion was on " Inflammatory Vascular Diseases affecting the Eye ", the three opening speakers being Mr. R. C. Davenport (*London*), Dr. S. P. Meadows (*London*), and Professor J. B. Duguid (*Newcastle-upon-Tyne*). Davenport took for his subject retinal vasculitis, with particular reference to its association with various forms of uveitis. Four features of this association had impressed him: the long interval which may separate the retinal and uveal inflammations, their occasional alternation or simultaneous appearance, the fact that the retinal changes may affect either arteries or veins, alone or together, and the occasional siting of a venous lesion entirely within the nervehead. Four cases illustrating these features were described. The first had developed bilateral iridocyclitis 20 years after an attack of bilateral retinal phlebitis. In the second, unilateral sclero-keratitis associated with cervical adenopathy had been followed, 15 years later, by retinal phlebitis in the same eye. In the third, intermittent attacks of retinal phlebitis, uveitis, and episcleritis had continued during 13 years. The fourth patient had suffered from mild anterior uveitis, some degree of retinal vasculitis and bilateral papillitis for 2 years. In every case all investigations were negative, showing that the cause of the disease remained a matter for further research. In conclusion, reference was made to the association of retinal vasculitis with lesions of the central nervous system, particularly disseminated sclerosis.

Meadows' theme, based on twelve personal cases, was " temporal arteritis and loss of vision ". After describing the distribution, incidence, and pathology of the disease, he outlined the clinical picture. There was often an initial period of malaise, anorexia, and slight fever, followed by headache and tenderness of the scalp; 40 per cent. of cases showed ocular signs of which the most common (25 per cent.) was blindness of rapid onset in one or both eyes, the second eye being affected at the same time as the first or a few days later. Ophthalmoscopy showed retinal arterial occlusion in a few instances, but, more commonly, only moderate papilloedema. Sometimes the fundi were normal. Other neurological changes such as hemiplegia and dementia occurred. The

diagnosis was confirmed by the nodular, tender, usually pulseless, temporal artery or arteries, and by temporal artery biopsy. The sedimentation rate was raised. The disease was self-limiting, lasting for 3 or 4 months, and though sight rarely recovered the general prognosis was surprisingly good. Treatment by anti-coagulants and cortisone had been disappointing, though the latter was, theoretically, the most hopeful.

Duguid discussed the aetiology of peri-arteritis nodosa. Consideration of the haphazard distribution of this disease and of the fact that in the kidney it caused infarction, had led him to the tentative conclusion that its lesions were caused by embolism or thrombosis. The objection to such a hypothesis was that histological sections of affected vessels frequently showed no evidence of such events. In answer to this, first, a small lesion was easy to miss in the histological examination of the course of a vessel. Secondly, as a series of personal experiments demonstrated, when an embolus or thrombus was lodged in a vessel it rapidly became covered by endothelium and incorporated in the vessel wall, so that its original nature was difficult to identify. Although the application of this theory to lesions affecting large vessels was not easy, in the case of small vessels there was no insuperable objection to it on histological grounds, and it did, in his opinion, offer the most satisfactory explanation of observed facts.

Papers were read by Mr. A. J. B. Goldsmith, evaluating the operation of cyclodiathermy; by Mr. George Black discussing the diagnosis of orbital tumours, and emphasizing the value of radiography after air injection into the orbit and his preference for transfrontal exploration rather than anterior biopsy when the tumour did not present anteriorly; by Dr. G. V. T. Matthews who demonstrated that migrant birds find their direction largely by the position and movements of the sun.

Films were shown by Professor H. M. Dekking on " Retinal Detachment ", by Dr. G. I. Scott on " Advancement and Resection of the Levator for Correction of Congenital Ptosis ", by Mr. A. G. Leigh on " Keratoplasty ", and by Mr. H. Vernon Ingram on " The Miner at Work ".

A symposium was held on " Changes in the Incidence of the Eye Affections of Coal-Miners in the last 25 Years ". Dr. Dorothy Campbell discussed miners' nystagmus, emphasizing the importance of accurate certification, of treatment, and of social welfare keeping the incidence down to a minimum. Mr. W. J. Wellwood Ferguson presented statistics on the incidence and nature of ocular injuries in mining with general reference to methods of mining and methods of treatment and prevention. Dr. G. I. Scott drew attention to the declining incidence of hypopyon ulcers in miners during the past 25 years, which could be attributed to the improved prophylactic treatment given in first-aid at the pit-head as well as by the general practitioner.

At a short symposium on " Gonioscopy after Glaucoma Operations ", Mr. H. E. Hobbs illustrated the gonioscopic appearance after drainage operations, and assessed the clinical effects of the anatomical variants shown, and Mr. Redmond Smith discussed the general interpretation of broad and narrow angles and the presence of peripheral anterior synechiae.

Further papers were contributed by Mr. Barrie Jones on " Acute Retinal Phlebitis with Infectious Mononucleosis "; by Mr. J. Stewart Gourlay on " Shagreen in Cataract Surgery ", suggesting that the presence of a good capsular shagreen indicated that the capsule was not likely to rupture on manipulation; by Mr. D. P. Greaves, on the " Technical Advances in Experimental Perforating Keratoplasty ", which now permitted a high proportion of clear grafts; by Mr. E. C. Glover on the " Statistical Analysis of the Results of Cataract Surgery at Moorfields in 1949 "; and by Mr. Alexander MacRae, who gave a follow-up of his earlier series of " Congenital Vascular Veils in the Vitreous " and discussed their significance. A film was shown by Professor R. C. Browne on the " Experimental Production of Nystagmus in Animals ".

Short demonstrations were given by Mr. A. Seymour Philps on " Keratography and its Value in Recording Irregularities of Corneal Curvature " (illustrated with a film by Dr. Hansell); by Mr. A. T. G. Evans, on " Essential Atrophy of the Choroid with Ataxia and a strong Family History of similar conditions "; by Mr. J. Frankenthal on " Large Cyst of the Iris following Lens Extraction "; by Mr. P. J. L. Hunter, on " Bilateral Lymphosarcoma of the Orbit "; by Mr. E. F. King, on " Secondary Carcinoma of the Choroid, which had shown a striking regression after Adrenalectomy", and by Mr. P. D. Trevor-Roper, on a Case in which symmetrical metastases from a breast carcinoma had presented in the angles of both anterior chambers, strictly confined to the horizontal axes, suggesting that this could most easily be attributed to anatomical vagary of the retrobulbar arteries by which the metastases were diverted into the long posterior ciliary arteries rather than the short posterior ciliary arteries.

Apart from the excellence of the papers, this was considered to be a most successful congress and the hospitality of the local members was overwhelming. The Annual Dinner was held at the Station Hotel on April 22, and the next evening a civic reception was held by the Lord Mayor of Newcastle-upon-Tyne at the Laing Art Gallery. Nearly seventy members took part in an expedition to the Roman Wall under the guidance of Professor Ian Richmond.

Ophthalmological Society of New Zealand (B.M.A.)

VIII Annual Conference, 1953

The Eighth Annual Meeting of the Ophthalmological Society of New Zealand was held in Dunedin on November 4–6, 1953, under the presidency of Mr. W. E. Carswell, F.R.C.S.

It was a pleasure to welcome two distinguished overseas guests, Prof. H. J. Weve, M.D., of the University of Utrecht, Holland, and Mr. A. Seymour Philps, F.R.C.S., of St. Bartholomew's Hospital, London, whose papers added considerably to the interest and value of the meetings. Twenty-four members of the New Zealand Society were present representing just over half of the total membership.

Papers were read by Professor Weve on the "Diathermic treatment of different forms of retinal detachment", and the "Origin and relationship of disinsertions, retinal cysts, folds, and retrolental fibroplasia". Mr. Philps spoke on "Ptosis" and "Anæsthesia". At a combined session with the Department of Neurology, Sir Charles Symonds, Sims Commonwealth Travelling Professor, gave a most interesting talk on "Primary carotid artery thrombosis". Professor J. H. Smirk and Dr. A. E. Doyle of the Department of Medicine contributed a paper on "Retinal changes in hypertension", and Mr. Anthony James spike on "A neuro-surgical approach to failing vision".

Papers were given by members of the Society on a variety of topics: "Retrolental fibroplasia in the Auckland district", "One hundred extractions with an erisophake", "Modified McLean suture", "Lens-induced uveitis and glaucoma", "Two historical ophthalmic cases", "Mandibulo-facial dystrophy", and "Convergence deficiency".

One evening was devoted to the showing of an extremely good series of films for which the Society was greatly indebted to the overseas visitors. The majority of the papers will be published as an Ophthalmic Supplement to the *New Zealand Medical Journal*.

At the Annual Dinner it was announced by the President that Prof. Weve and Mr. Philps had both been elected Honorary Members of the Society. Dr. J. S. Munro of Palmerston North was elected President for the ensuing year. It is expected that the next (ninth) Annual Meeting will be held in Palmerston North in November, 1954.

Treacher Collins Prize, 1954

Dr. Robert Leishman of Glasgow has been awarded the Treacher Collins Prize for 1954 by the Ophthalmological Society of the United Kingdom for his essay on "The Eye in General Vascular Disease: Hypertension and Arteriosclerosis".

Percival J. Hay Memorial Prize, 1954

At the Annual Meeting of the North of England Ophthalmological Society at the Royal Infirmary, Sheffield, on May 29, 1954, the first Percival J. Hay Memorial Prize, value £60, was awarded to Mr. A. Stanworth, M.B., Ch.B., D.O.M.S., for his paper on "Science and Ophthalmology".

Dr. C. S. Swan has been appointed Honorary Assistant Ophthalmologist at the Royal Adelaide Hospital, South Australia.

The Gonin Oration at the University of Lausanne was given by Sir Stewart Duke-Elder on May 19, 1954.

OBITUARY

Leslie Gemmill Scoular

The Editorial Committee regret to announce the death of Dr. L. G. Scoular, M.D., D.O.M.S., which occurred on May 6, 1954.